

Review Article

The Link Between Culture, Cuisine, And Cancer (A Nigerian Perspective)

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Abstract:

Background: Cancer is a public health challenge in Nigeria, with cases rising in recent times. Research shows that dietary patterns play a substantial yet underestimated role in cancer incidence and death. Some Nigerian diets, which feature high consumption of red and processed meats, deep-fried foods, and the use of plastic bags when preparing, have been identified as culprits in the increased exposure to dietary carcinogens and elevation of cancer risk. Foods such as fruits, vegetables, and whole grains possess cancer-protective properties; however, their intake remains inadequate in combating diet-related cancers.

Aim: This review examines the relationship between diet and cancer in Nigeria, focusing on the current cancer burden of Nigeria, carcinogenic risks linked to food preparation, and preventive nutritional strategies.

Methods: Information was sourced from PubMed, Scopus, Google Scholar, and ResearchGate, alongside reports from global health agencies. Search terms included "diet," "cancer," "Nigeria," "food," "carcinogens," and "prevention." Only articles written in English language and that met predefined inclusion criteria were included.

Results: Evidence suggests that the consumption of carcinogens formed through traditional cooking methods increases the risk of cancer. Notable concerns include barbecue-style cooking, repeated use of cooking oils, and heavy metal contamination in local alcoholic beverages. Protective effects were observed in diets rich in fruits and vegetables.

Conclusion: Dietary practices in Nigeria significantly shape cancer risk. Targeted interventions promoting nutrition education, protective food intake, and safer cooking methods are essential to reduce carcinogen exposure and lower the national cancer burden.

Keywords: Diet; Cancer; Nigeria; Dietary Carcinogens; Toxins

Introduction

With a steady rise in both incidence and mortality rates, cancer has emerged as a major public health challenge in Nigeria (1). Among the various risk factors implicated in cancer development, diet stands out as a significant yet often overlooked contributor (2). In Nigeria, food is not merely a source of sustenance—it serves as a profound expression of culture, identity, and tradition. For generations, food has played a central role in fostering community bonds and reinforcing cultural values (3). However, the ingredients and preparation methods of many beloved Nigerian dishes may carry hidden health risks that silently contribute to cancer development.

While many foods are naturally nutritious, their preparation methods often turn them into sources of carcinogenic exposure. For example, the open-flame grilling of meat (suya - charred spiced meat), which involves direct contact with fire, can produce polycyclic aromatic hydrocarbons (PAHs). Similarly, deep-frying snacks like akara and puff-puff can lead to higher levels of acrylamide, a recognized carcinogen. These harmful compounds are by-products of high-temperature cooking methods that are widely used but rarely

scrutinized (4, 5). Smoked fish, (6) mouldy groundnuts and maize (contaminated with aflatoxins), (7) deep-fried street foods (e.g., akara, puff-puff), (8) and locally brewed alcohol (ogogoro) (9, 10), have also been linked with increased cancer risks due to contamination with carcinogenic compounds like PAHs, acrylamides, methanol, and aflatoxins. In addition, the repeated use of cooking oil for frying and high intake of salt-cured foods like stockfish and dried meats may further increase the risk of cancer, especially in a population already challenged by limited screening and dietary awareness. Diets high in fruits, vegetables, and essential micronutrients, such as vitamin C and folate, are associated with a lower risk of cancer. (9).

This article explores the connection between diet, cultural food practices, and cancer risk in Nigeria, emphasizing how evolving cooking traditions and modern dietary transitions may silently accelerate the nation's cancer burden. By critically examining the links between diet and carcinogenesis, we hope to illuminate a path toward culturally grounded, diet-based cancer prevention strategies.

Methodology

A comprehensive literature search was conducted using various databases, including PubMed, ResearchGate, Google Scholar, and a direct Google search, supplemented by grey literature from the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) reports. Keywords include diet, nutrition, cancer, Nigeria, dietary carcinogens, red and processed meat, polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (HCAs), toxins, and metals. The search strategy used combinations of keywords and Boolean operators, including: (diet OR nutrition) AND cancer AND Nigeria; (PAH OR HCAs) AND cancer AND Nigeria; food carcinogens OR cooking methods AND cancer. Only articles written in the English language were considered. EndNote 2025 was used to manage the references received from the search throughout the entire writing and review process. Two researchers independently screened the search results based on the title and abstract, and the other authors helped confirm eligibility based on the inclusion and exclusion criteria.

The authors read through the following sections: title, abstract, material and methodology, results, and discussion, to look for information of interest.

Inclusion criteria were:

1. Articles published in English from 2000 to 2025;
2. Studies focusing on diet-related carcinogens, nutritional epidemiology, or public health interventions relevant to Nigeria or Sub-Saharan Africa;
3. Reports providing quantitative or qualitative data on dietary patterns, cooking practices, or associated cancer risks.

Exclusion criteria were:

Studies unrelated to human cancer; 2. Articles lacking verifiable references or published in non-peer-reviewed outlets (except for authoritative agency reports). Given the heterogeneity of study designs and outcomes, a “narrative synthesis” approach was adopted, integrating biochemical, epidemiological, and cultural perspectives without quantitative meta-analysis.

Cancer Burden in Nigeria

As of July 9, 2025, data from the World Health Organization (WHO) indicated that Nigeria had a cancer burden of 269,109 prevalent cases (over a 5-year period), 127,763 new cases, and 79,542 deaths in 2022. Prostate, colorectal, and liver cancers were the top three

leading cancers among males, while breast, cervix uteri, and colorectal cancers were the most common among females (11). This represents a marked increase compared to the 102,079 cases documented in 2012 (12), indicating a rising cancer burden in the country. When

compared to other diseases, cancer, alongside other non-communicable diseases (NCDs), was responsible for up to 27% of deaths in Nigeria in 2019, and caused 565 deaths per 100,000 in males and 546 deaths in females in 2021. This shows that cancer is not an isolated problem but part of a larger NCD crisis in Nigeria. This burden is made worse by Nigeria's fragile healthcare infrastructure and a critical shortage of skilled health workers. With a density of only 1.83 skilled health workers per 1,000 people, Nigeria falls

significantly short of the WHO's recommended minimum of 4.45 per 1,000. This deficit is further strained by the increasing emigration of medical professionals and a projected population surge to about 263 million by 2030, suggesting that the country's cancer control efforts will face mounting challenges unless urgent investments in healthcare human resources are made (13). Table 1 shows the cancer burden in Nigeria based on World Health Organization data for 2022.

Table 1. Cancer Burden in Nigeria – Statistics at a Glance (2022)

Metric	Males	Females	Both Sexes
Population	109,872,878	106,874,055	216,746,933
Number of New Cancer Cases	48,096	79,667	127,763
Age-Standardized Incidence Rate	93.9	133.3	113.6
Cumulative Risk of Developing Cancer (% < 75 yrs)	10.4	13.8	12.1
Top 3 Leading Cancers (by new cases)	Prostate, Colorectum, Liver	Breast, Cervix uteri, Colorectum	Breast, Prostate, Cervix uteri
Number of Cancer Deaths	32,905	46,637	79,542
Age-Standardized Mortality Rate	68.4	81.4	74.6
Cumulative Risk of Dying from Cancer (% < 75 yrs)	7.4	8.7	8.0
Top 3 Leading Cancers (by deaths)	Prostate, Colorectum, Liver	Breast, Cervix uteri, Colorectum	Breast, Prostate, Cervix uteri
5-Year Prevalent Cases	94,487	174,622	269,109

Data from the World Health Organization (2022) shows the cancer burden of Nigeria over 5 years, with breast and prostate cancer leading the mortality for women and men, respectively (11)

Cancer-Linked Substances Found in Traditional Nigerian Dishes

Heterocyclic Amines (HCAs): Heterocyclic amines are a class of carcinogenic and mutagenic compounds formed during the cooking of muscle meats such as beef, pork, poultry, and fish at high temperatures. These compounds are produced through the reaction of amino acids and creatine or creatinine when meat is grilled, fried, or otherwise cooked using methods involving intense heat. HCAs are generated in significantly higher quantities when meats are overcooked, blackened, or charred, as is commonly observed in local delicacies such as suya. To date, more than 25 HCAs have been identified, with seventeen specifically formed from the cooking of muscle meats. Among these, the International Agency for Research on

Cancer (IARC) has classified four HCAs—IQ, MeIQ, MeIQx, and PhIP—as probable or possible human carcinogens. The carcinogenic potential of HCAs is significantly enhanced after metabolic activation by cytochrome P450 1A2-mediated oxidation, followed by acetylation or sulfation, which transforms these compounds into reactive mutagens capable of forming DNA adducts and inducing genetic mutations that initiate carcinogenesis (14). HCAs have been implicated in various cancer types, such as colon, breast, and prostate cancer (15). Given the popularity of grilled meat in Nigeria, understanding the role of HCAs in diet-related cancer (16, 17). The structural formula of HCAs is given in Figure 1.

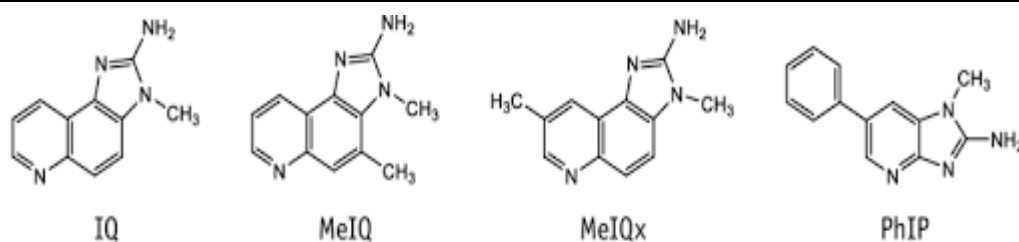


Fig. 1. Structural formula of four heterocyclic Amines (HCAs). "Source: (18)"

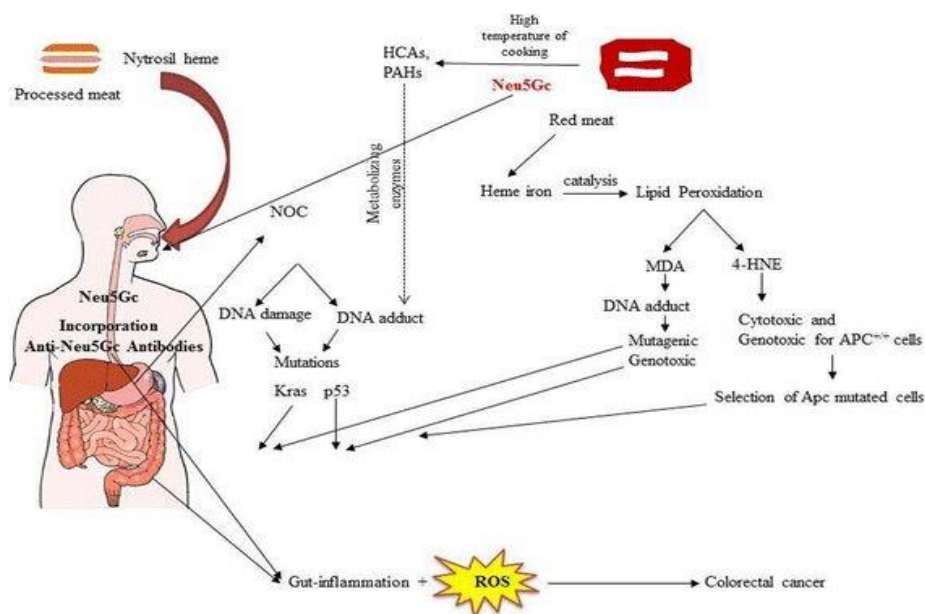


Fig. 2. Mechanisms underlying the carcinogenesis of PAHs and HCAs in colorectal cancer. PAHs and HCAs are produced in significant amounts when red meat is cooked at high temperatures. PAHs and HCAs, when present in the human body, are metabolized by phase I enzymes such as cytochrome P450 monooxygenases (CYP) and phase II enzymes and eventually bind to DNA, forming a DNA adduct. The formation of these DNA adducts results in DNA mutations, alteration of gene expression profiles, and tumorigenesis. Furthermore, heme produced from red meat by lipid peroxidation and endogenous formation of N-nitroso compounds (NOCs) can provoke several DNA mutations responsible for colorectal cancer. Lastly, the association of products of lipid peroxidation (induced by heme), reactive oxygen species (ROS) present in red and processed meat, and N-glycolylneuraminic acid (Neu5GS) would result in inflammation, which can lead to the development of colorectal cancer. "Source: (19, 20)"

Suya (Red meat): The preparation method of suya, which is a traditional Nigerian grilled meat delicacy, has been linked to the formation of carcinogenic compounds, particularly heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) (21). Studies have shown that grilling meat over open charcoal flames, as commonly practiced in suya preparation, facilitates the production of HCAs such as PhIP, MeIQ, and MeIQx, which form when creatine and amino acids in meat react at high temperatures (22). These HCAs, along with PAHs formed when fat drips onto hot coals and produces smoke, are classified as probable human carcinogens by the International

Agency for Research on Cancer (IARC) (23, 24). Aside from the presence of HCAs and PAHs in suya, which pose health risks, a study by Oyewole et al. (2024) identified dangerously high levels of arsenic, chromium, and cadmium in suya samples sold across Ibadan, Nigeria. These heavy metals exceeded safety thresholds and were associated with incremental lifetime cancer risk (ILCR) values above acceptable limits (25).

Polycyclic Aromatic Hydrocarbons (PAHs):

Polycyclic aromatic hydrocarbons (PAHs) are compounds composed of multiple fused aromatic rings, which are primarily formed through the incomplete combustion of organic materials, such as wood, fossil fuels, and biomass. They represent one of the most concerning classes of environmental carcinogens linked to diet-related cancer risk. Although PAHs are naturally occurring, they can also be formed during food processing methods such as grilling, roasting, drying, and smoking. From a toxicological standpoint, PAHs are not inherently carcinogenic in their native state. However, upon entering the body, these compounds undergo metabolic activation, particularly in the liver, where cytochrome P450 enzymes transform them into reactive epoxide intermediates. These electrophilic metabolites, such as PAH-derived diol epoxides, can form covalent adducts with nucleophilic sites on DNA. This process results in

mutations that can initiate the development of cancer. PAHs have been implicated in various cancer types, such as lung, skin, and bladder cancer (26). In Nigeria, dietary exposure to PAHs is of concern due to cultural cooking practices. The preparation of popular foods such as suya (spiced, flame-grilled meat), smoked fish, and roasted plantains typically involves direct flame or smoke contact, which are conditions that favor PAH formation (27-30). Four representative examples of PAHs are shown below in Figure 3.

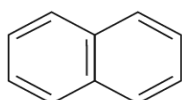
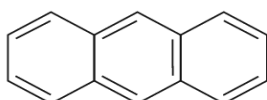
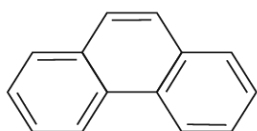
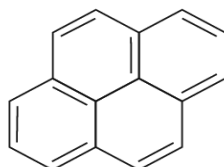
Naphthalene**Anthracene****Phenanthrene****Pyrene**

Fig. 3. Chemical structure of four polycyclic aromatic hydrocarbons (PAHs). Source: (31)."

Kilishi: Kilishi, a popular sun-dried and spiced meat delicacy in Nigeria, was found to contain concerning levels of PAHs when processed using traditional smoking techniques. A study by Fakolade et al. (2017) revealed that commercially prepared Kilishi samples contained PAH concentrations as high as 8.80 µg/kg, exceeding the European Commission's safety threshold of 5.0 µg/kg for benzo[a]pyrene, a marker for total PAH contamination. Similarly high levels (6.40 µg/kg) were also detected in laboratory-smoked samples. These elevated values are largely attributed to direct exposure to smoke from coal combustion at high temperatures (220–250 °C) and the use of oily ingredients like groundnut paste, which increase PAH accumulation through pyrolysis. Since PAHs are mutagenic and carcinogenic compounds, their presence in traditionally smoked Kilishi raises significant public health concerns. Routine consumption of such contaminated products could contribute to long-term cancer risk, particularly cancers of the gastrointestinal tract (32).

Smoked fish or meat (e.g., barbecue): Barbecue cooking has been strongly linked to the formation of PAHs, compounds known for their carcinogenic potential (33, 34). A study by Sumer and Oz (2023) investigated the effects of direct and indirect barbecue

cooking on beef. The results revealed that while cooking method had no significant effect on total PAH4 or PAH8 levels, direct barbecuing and higher cooking degrees (well-done) led to increased levels of benzo[a]pyrene (BaP) and dibenzo[a,h]anthracene (DahA), both highly toxic PAHs. Cooking loss and lipid oxidation also increased significantly with more intense cooking, indicating both nutritional degradation and the formation of higher toxic compounds (35). An Earlier study by Rose et al. (2015) studied PAH formation during various home-cooking methods, including barbecuing with and without wood chips. The study showed that grilling, frying, toasting, and roasting produced minimal PAHs, but barbecuing—especially with charcoal and wood chips—led to significant PAH accumulation in meats like beef and sausages. This study also revealed that BaP levels in heat-treated meat products frequently exceeded the European Commission's safety threshold of 5.0 µg/kg (36). These studies' findings indicate that barbecue techniques, particularly direct flame cooking with charcoal, elevate PAH exposure and may increase cancer risk.

Phthalates: Phthalates are chemicals that are commonly used in plastic production for various items (37). They are considered possible human carcinogens based on evidence from several studies (38). Phthalates such as di(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP) have shown hepatocarcinogenic effects in rodents, primarily through activation of PPARα, a pathway linked to liver tumor formation (39). DEHP, in particular, has been associated with liver, testicular, prostate, and breast cancers in both in vivo and in vitro models. Lifelong exposure to DEHP in male rats led to increased liver and testicular tumor development, while its metabolite MEHP has been shown to promote prostate and breast cancer cell proliferation. Prenatal and lactational exposure to DEHP also increased susceptibility to prostate tumors in male offspring. In breast cancer models, DEHP stimulated tumor growth without inducing apoptosis, and co-exposure with bisphenol A further enhanced mammary tumor risk and reduced latency (40). Phthalates are also endocrine disruptors, capable of mimicking hormones and interfering with reproductive health and development. Some studies suggest phthalates promote breast tumor growth via estrogen receptor (ER) signaling, while others report estrogen-independent and antiestrogenic mechanisms (14). Figure 4 and Figure 5 illustrate the chemical structures of commonly used phthalates.

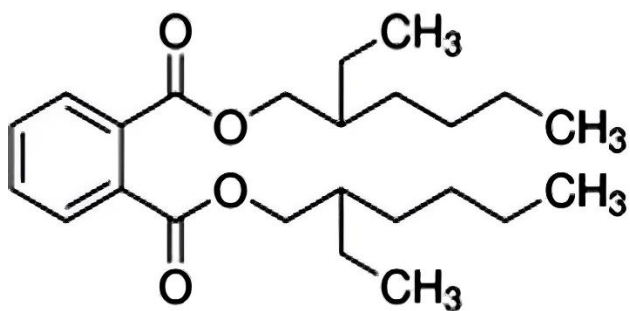


Fig. 4. Structure of di-(2-ethylhexyl) phthalate (DEHP) used in the production of building products (wallpaper, wire and cable insulation), car products, clothing (footwear, raincoats), food packaging, etc. "Source: (41)."

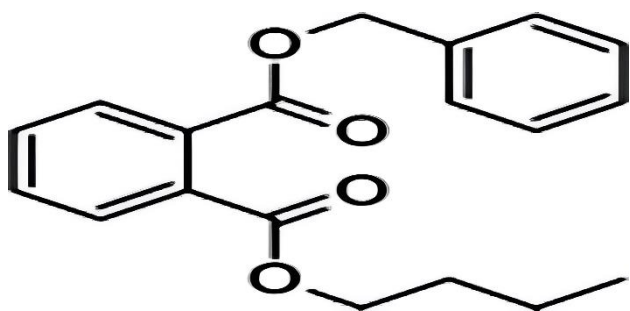


Fig. 5. Structure of benzyl butyl phthalate (BBP), used in the traffic cones, food conveyor belts, and artificial leather. "Source: (41)."

Moi Moi: Moi Moi is a steamed bean paste dish typically prepared from blended cowpeas mixed with seasonings and various other ingredients (42). Moi Moi, as a dish, doesn't pose any danger to health, but the method of cooking is where the problem lies. A study by Iguh et al. (2023) found that cooking Moi Moi in polyethylene film (nylon) led to the migration of di-butyl, di-2-ethylhexyl, and benzylbutyl phthalates into the food. These phthalates were not detected in the raw paste but appeared after cooking and increased further after three days of frozen storage and reheating. This migration occurs because phthalates are not chemically bonded to the plastic, allowing them to leach under

high heat. Although detected levels were below safety limits, their presence raises concern due to the carcinogenic and endocrine-disrupting potential of phthalates (43).

Acrylamides: Acrylamide is a chemical monomer commonly used in producing plastics, dyes, adhesives, and food packaging. However, since 2002, it has gained attention as a food contaminant formed unintentionally during high-temperature cooking methods such as frying, baking, and roasting. Carbohydrate-rich foods like fried potatoes, coffee, biscuits, and bread are notable sources. Acrylamide forms through the Maillard reaction—a chemical interaction between asparagine (an amino acid) and reducing sugars, especially under conditions of high heat and low moisture (44, 45). This compound poses significant health risks. The International Agency for Research on Cancer (IARC) classifies acrylamide as a probable human carcinogen. Mechanistic studies show that it is genotoxic, forming glycidamide, a metabolite that binds to DNA and proteins, promoting mutations. Beyond its cancer risk, acrylamide has been associated with neurotoxicity and reproductive toxicity in animal models (46). In Nigeria, the rise in consumption of fried and baked street foods—such as puff-puff, buns, and yam fries—may increase acrylamide exposure, especially in urban areas. The structural formula of acrylamide is presented in Figure 6, while its metabolic pathway is shown in Figure 7.

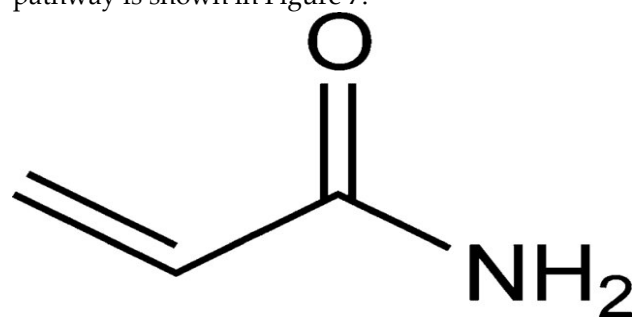


Fig. 6. Structural formula of acrylamide. "Source: (47)."

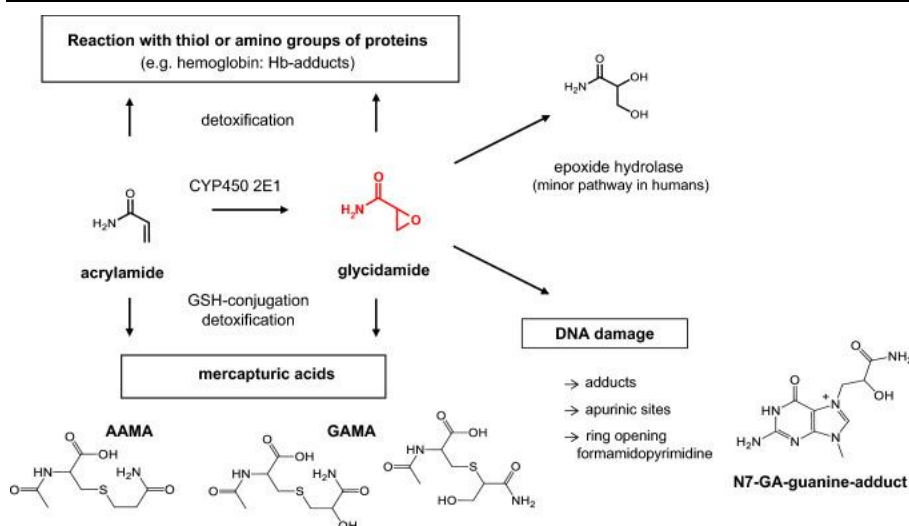


Fig. 7. Metabolic pathway of acrylamide in carcinogenesis.

Once ingested and absorbed from the gastrointestinal tract, acrylamide is converted by cytochrome P450 2E1 (CYP450 2E1) into the genotoxic epoxide glycidamide. Glycidamide, in turn, forms DNA adducts, primarily at N7 of guanine (N7-GA-Gua). This interaction of glycidamide with DNA is what is considered to represent the key event resulting in genotoxicity and carcinogenicity of acrylamide. "Source: (48)."

Puff puff and Akara: Puff-puff and akara are two widely consumed West African fried foods, but research has shown that they contain varying levels of acrylamide, which associates them with potential cancer risks. A study by Akinosun et al. (2025) (49) found that frying puff-puff and akara instead of baking resulted in a 4.40% and 8.09% increase in acrylamide, respectively. In this study, temperature and cooking time were 2 critical factors that influenced acrylamide formation. This study also indicated that an increase in cooking temperature from 150 °C to 180 °C resulted in a 157.63% rise in puff-puff and a 227.78% increase in akara acrylamide levels. Further heating to 210 °C produced an additional 21.51% (puff-puff) and 11.2% (akara) rise. These findings emphasized that higher temperatures and longer durations significantly elevate acrylamide content (49). To minimize health risks, the authors recommend processing puff-puff and akara at temperatures below 150 °C and for less than 5 minutes. When this is not feasible, cooking should not exceed 15 minutes to limit acrylamide accumulation.

Trans fat: Trans fatty acids (TFAs), also known as trans fats, are a type of unsaturated fat with at least one non-conjugated double bond in the trans configuration, as defined by Regulation (EU) No 1169/2011 (50). While small amounts occur naturally in meat and dairy products from ruminants, the primary dietary source is industrially produced partially hydrogenated oils, which are used in processed and deep-fried foods. (51) TFAs have been implicated in the development of several malignancies, notably breast and colorectal cancers. Their consumption disrupts

lipid metabolism, promotes systemic inflammation, and increases oxidative stress, conditions conducive to initiating carcinogenesis (52, 53). Emerging research suggests that trans fats may influence hormonal pathways, potentially contributing to hormone-related cancers. Beyond cancer, TFAs are associated with other chronic conditions, including cardiovascular disease, diabetes, and pregnancy-related complications, which may indirectly affect cancer susceptibility (52). Figure 8 below shows the mechanism of action of trans fat in carcinogenesis.

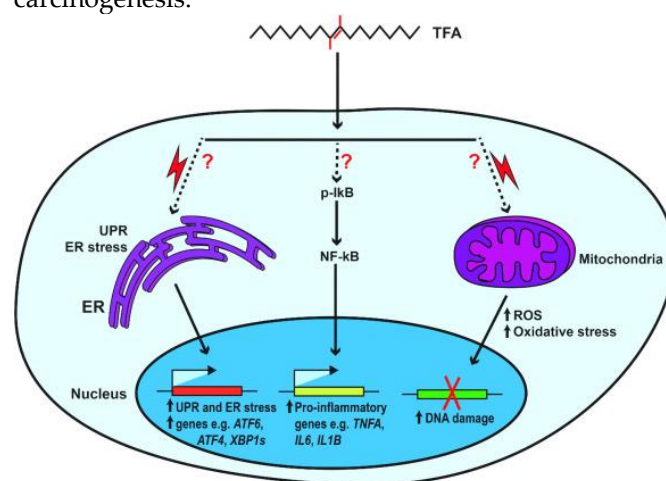


Fig. 8. The mechanism of action of trans fat in carcinogenesis. Trans fat may stimulate inflammation through the induction of NF-κB signaling and endoplasmic reticulum stress. Trans fat can also induce reactive oxygen species (ROS) production, which leads to DNA damage. Inflammation and DNA damage both contribute to cancer development. "Source: (54)."

Fried plantain: Fried plantain, a popular deep-fried dish across West Africa, particularly in Ghana and Nigeria, has become a dietary staple. However, recent evidence has raised concerns about their safety due to the presence of trans fatty acids (TFA). A study by Addo et al. (2023) found significant levels of TFAs in plantain chips sold in Accra, Ghana, especially trans isomers of linoleic acid. These harmful fats were linked

to poor frying practices, with 97% of vendors reusing oil multiple times (55).

Heavy Metals: Heavy metals such as chromium (Cr), nickel (Ni), manganese (Mn), and iron (Fe) are environmental contaminants known for their toxicity and carcinogenic potential. Due to their non-biodegradable nature, they accumulate in tissues and are linked to oxidative stress, organ dysfunction, and cancer (56, 57). A study by Ogidi et al. (2024) analyzed

seven locally produced Nigerian beverages and found that Cr, Ni, Mn, and Fe levels in many samples exceeded WHO limits. While non-carcinogenic risk values were below hazard thresholds, carcinogenic risk values for several metals surpassed the acceptable 1×10^{-4} limit, suggesting long-term health hazards (10). Figure 9 illustrates the mechanisms through which heavy metals contribute to carcinogenesis.

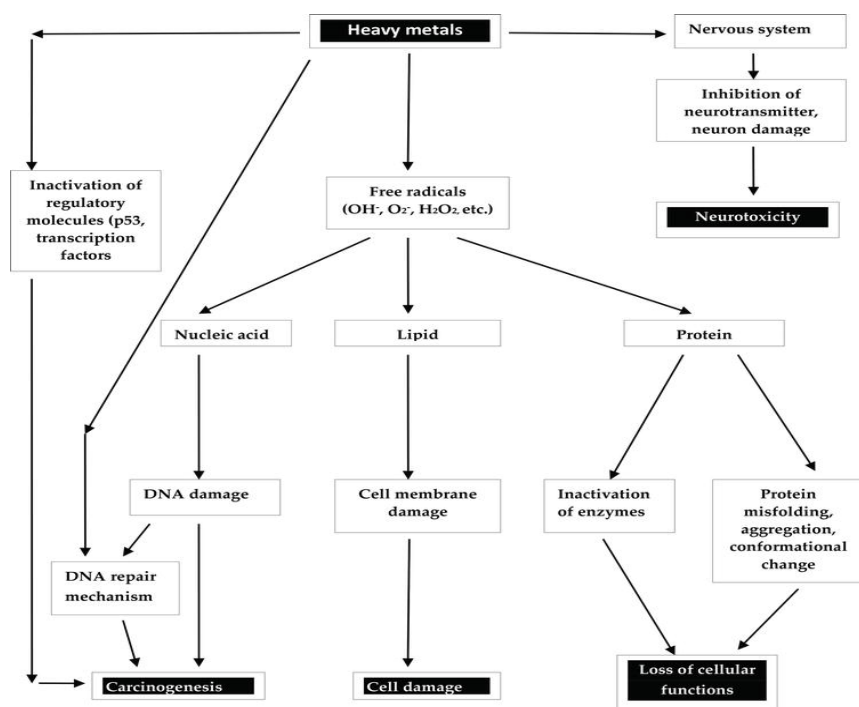


Fig. 9. Mechanism through which heavy metals cause toxicity and contribute to carcinogenesis. "Source: (58)"

Ogogoro: Ogogoro, a distilled spirit derived from fermented *Raphia* palm sap, is consumed widely across southern Nigeria. The study by Ogidi et al. (2024) found that while chromium and nickel levels in ogogoro were within WHO limits, iron levels (1.115 mg/kg) exceeded the safe threshold (0.3 mg/kg). Excess iron has been implicated in the generation of reactive oxygen species (ROS), leading to oxidative damage,

cellular dysfunction, and increased cancer risk, particularly in the liver and pancreas. Although the non-carcinogenic risk was low, the iron-related carcinogenic risk was elevated (10).

Mokite: Mokite, a relatively new marijuana-infused beverage, showed dangerously high levels of iron (1.83 mg/kg) and copper (0.66 mg/kg). The carcinogenic risk values for multiple metals exceeded the acceptable limit. Iron toxicity from Mokite may impair liver function and catalyze ROS production, leading to DNA damage and mutagenesis (10).

Strategies for Cancer Prevention Through Diet

Food regulation: Food regulation by governmental health agencies is important in minimizing cancer risk due to carcinogen exposure. In Nigeria, governmental health regulatory bodies such as the National Agency for Food and Drug Administration and Control (NAFDAC) and the Standards Organization of Nigeria (SON) are tasked with overseeing food safety and creating health policies. Still, the enforcement of these policies remains limited, particularly in informal markets and street food settings where harmful practices like excessive frying, smoking

with firewood, and use of illegal preservatives are common. Not much attention is given to the regulation of known carcinogens such as PAHs, acrylamide, mycotoxins, and heavy metals, all of which are prevalent in local diets through grilled meats, fried snacks, improperly stored grains, and unregulated alcoholic beverages. Strengthening food regulation, educating food handlers on safe preparation techniques, and enlightening the public on healthier food choices should be the priority of these organizations.

Public Health Education: Some diet puts people at risk of cancer; other diets help prevent the risk of cancer (59). The Mediterranean diet is a dietary recommendation that emphasizes eating plant-based foods and healthy fats (60). This diet is characterized by a low saturated fat content, high monounsaturated fat intake (primarily from the high consumption of olive oil), and an abundance of dietary fiber. It includes vegetables, fresh fruit, legumes, non-refined cereals, nuts, poultry, a moderate amount of seafood and dairy products, low intake of red and processed meat, and moderate alcohol consumption. It is rich in antioxidant

compounds and bioactive elements with anti-inflammatory effects, and it has a low glycemic index. Research has shown that adherence to the Mediterranean diet has been associated with decreased risk of several chronic diseases, such as cardiovascular disease, diabetes, neurodegenerative diseases, and cancer (61, 62). As shown in Table 2, the Mediterranean diet emphasizes anticarcinogenic foods such as fruits, vegetables, whole grains, and healthy fats, whose bioactive compounds play protective roles against cancer.

Table 2. Anticarcinogenic foods and their main components

Food/Fruit	Main component	Mechanism of action	Reference
Banana	Flavonoid, phenolics	Inhibits ROS, inhibits cell cycle, and promotes apoptosis.	(63, 64)
Avocado	Triterpenoids, polyphenols, carotenoids, glutathione	Induce cell death, inhibit proliferation, and target the cell cycle	(65-67)
Apple	Triterpenoids, Kaempferol (flavonoid), quercetin	Antioxidant effects induce apoptosis	(68, 69)
Paw paw (papaya)	Acetogenin, alkaloids, terpenoids	Block ATP production, thereby inducing apoptosis and cell cycle arrest	(70, 71)
Pomegranate	Ellagitannins	Inhibit cancer cell proliferation, induce apoptosis, and disrupt signaling pathways.	(72-74)
Grapes	Proanthocyanidins, phenolic antioxidants, kaempferol, and quercetin	Inhibition of cell proliferation, induction of cell cycle arrest in the G1 phase, and apoptosis	(75-79)
Citrus fruits (oranges, lemons, grapefruits)	Naringin (flavonoid)	Inhibiting cancer cell growth, inducing apoptosis (programmed cell death), and modulating various signaling pathways	(80-82)
Berries (blueberries, raspberries, strawberries)	Flavonols, anthocyanins (ACNs), proanthocyanidins (PACs), and phenolic acids	Reduce and repair damage resulting from oxidative stress and inflammation	(83, 84)
Cruciferous vegetables (broccoli, cabbage, kale, Brussels sprouts)	Isothiocyanates, phenolic compounds, flavonoids	Block DNA replication in noncancerous cells, inhibit the binding of carcinogens to DNA, reduce angiogenesis, and increase apoptosis.	(85)
Tomatoes	Lycopene	Antioxidant effect inhibits cancer cell proliferation	(86, 87)
Garlic & Onions	Allicin and sulfur compounds	DNA damage protection, induction of cell death (apoptosis), inhibition of cell proliferation, and blocking of angiogenesis and metastasis	(88-93)
Carrots	B-carotene (carotenoids) and chlorogenic acid (CHA)	Antioxidant effect, inhibiting cell proliferation, inducing apoptosis, and suppressing tumor growth.	(94-96)
Chili pepper	Capsaicinoids (alkaloids)	Initiate apoptosis, cell growth arrest, and suppress angiogenesis and metastasis.	(97, 98)
Turmeric	Curcumin	Suppress cell growth and proliferation, mitigate DNA damage	(99-101)
Whole grains	Dietary fibers	Promote apoptosis, dilute carcinogens, antioxidant effect, and anti-inflammatory effect.	(102, 103)
Legumes (beans, lentils, chickpeas)	Fiber, tannins, flavonoid (quercetin), saponins	Increase growth arrest and apoptosis, antioxidant effect	(104, 105)
Nuts (cashew nuts, walnuts, peanuts)	Alkaloids, polyphenols	Arrest the cell cycle, prevent microtubule formation	(106-108)
Olive oil	Oleuropein (phenols)	Promote apoptosis	(109-112)

Conclusion

The delicate relationship between diet, culture, and cancer in Nigeria highlights an urgent need for multidisciplinary public health intervention. While diets are deeply rooted in the fabric of Nigerian identity, evolving dietary habits—marked by the increasing consumption of fried foods, smoked meats, unregulated alcoholic beverages, and poorly stored grains—pose significant carcinogenic risks. These risks are compounded by socioeconomic challenges, lack of food safety enforcement, and limited public awareness. For the country to overcome the danger posed by diet-related cancers, it must prioritize a comprehensive national strategy that integrates cancer prevention into food systems, health education, and regulatory frameworks. Public enlightenment campaigns should focus on promoting safer cooking methods, recognizing hazardous food additives, and encouraging a shift towards more plant-based, fiber-rich diets. Regulatory bodies must be empowered to monitor and control food contaminants such as PAHs, acrylamide, heavy metals, and mycotoxins, particularly in informal markets and local food production chains.

Recommendation:

The authors recommend establishing a National Dietary and Cancer Prevention Task Force, comprising oncologists, nutritionists, food scientists, policymakers, and community leaders. This body should guide the

formulation of culturally appropriate dietary guidelines, oversee food safety reforms, promote local natural and healthy diets, and champion nutrition education across all levels. Only through such a collaborative and culturally sensitive approach can Nigeria effectively reduce the burden of cancer linked to dietary and lifestyle factors. The Ministry of Health in Nigeria and other associated bodies should encourage, assist, and fund research that produces data on the percentage of cancers in Nigeria attributed to dietary risk, as well as strategies for reducing the cancer burden in Nigeria.

Strengths and Limitations.

This review study addresses an important but underexplored topic using the most recent data and provides practical solutions to combat the problems highlighted in the study. Nonetheless, there are limitations, which include reliance on secondary sources of data; underrepresentation of protective foods in the Nigerian (local) context; the narrative review approach instead of a systematic review pattern; and scarcity of local dietary and cancer data. Despite these challenges, the review offers a valuable foundation for future research and culturally relevant interventions to reduce diet-related cancer risks in Nigeria.

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References

1. Fatiregun O. Demographic and Population Determinants, and Consequences of Cancer Incidence and Survival in Nigeria. In: Wotela K, editor. Historical and Contemporary Demographic and Population Issues in Africa and South Asia. Rijeka: IntechOpen; 2025.
2. Rodríguez-Molinero J, Migueláñez-Medrán BDC, Puente-Gutiérrez C, Delgado-Somolinos E, Martín Carreras-Presas C, Fernández-Farhall

- J, et al. Association between Oral Cancer and Diet: An Update. *Nutrients*. 2021;13(4). DOI: <https://doi.org/10.3390/nu13041299>
3. Uro-Chukwu H. Food culture, lifestyle & traditional food festivals: a narrative on the public health relevance of the traditional yam recipes in the Afikpo New Yam Festival. *World Nutrition*. 2024;15:66-79.
4. Başaran B, Çuvalcı B, Kaban G. Dietary Acrylamide Exposure and Cancer Risk: A Systematic Approach to Human Epidemiological Studies. *Foods*. 2023;12(2). DOI: <https://doi.org/10.3390/foods12020398>
5. Oyewole O, Balogun D, Olufisayo I, Ajao T, Niyi O, Alao A-Z, et al. QUALITY ASSESSMENT AND SAFETY OF COMMERCIALY SOLD STEAK MEAT "SUYA" IN IBADAN METROPOLIS: A MENACE TO PUBLIC HEALTH. 2024;8:120-9.
6. Iko Afé OH, Kpoclou YE, Douny C, Anihouvi VB, Igout A, Mahillon J, et al. Chemical hazards in smoked meat and fish. *Food Sci Nutr*. 2021;9(12):6903-22. DOI: <https://doi.org/10.1002/fsn3.2626>
7. Ilesanmi FF, Ilesanmi OS. Knowledge of aflatoxin contamination in groundnut and the risk of its ingestion among health workers in Ibadan, Nigeria. *Asian Pac J Trop Biomed*. 2011;1(6):493-5. DOI: [https://doi.org/10.1016/S2221-1691\(11\)60108-1](https://doi.org/10.1016/S2221-1691(11)60108-1)
8. Stott-Miller M, Neuhouser ML, Stanford JL. Consumption of deep-fried foods and risk of prostate cancer. *Prostate*. 2013;73(9):960-9. DOI: <https://doi.org/10.1002/pros.22643>
9. Mohammed HA, Sulaiman GM, Al-Saffar AZ, Abomughaid MM, Hussein NN, Salih ZT, et al. Roles of foods and related components: an overview on cancer causatives, and plausible preventions. *Applied Food Research*. 2025;5(2):101132. DOI: <https://doi.org/10.1016/j.afres.2025.101132>
10. Ogidi OI, Enenebeaku UE, Okara E, Elumelu SA. Toxic Metal Profiles, Carcinogenic and Non-Carcinogenic Human Health Risk Assessment of Some Locally Produced Beverages in Nigeria. *Journal of Toxicology and Risk Assessment*. 2021;7:039.
11. Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, et al. Global Cancer Observatory: Cancer Today Lyon, France: International Agency for Research on Cancer; 2024 [2025 Jul 9]. Available from: <https://gco.iarc.who.int/today>.
12. Morounke SG, Ayorinde JB, Benedict AO, Adedayo FF, Adewale FO, et al. Epidemiology and Incidence of Common Cancers in Nigeria. *Journal of Cancer Biology & Research*. 2017;5(3):1105.
13. Olatunji G, Aderinto N, Kokori E, Abraham IC. Nigeria's new policy: solution for the health-care workforce crisis? *The Lancet*. 2024;404(10460):1303-4. DOI: [https://doi.org/10.1016/S0140-6736\(24\)00781-1](https://doi.org/10.1016/S0140-6736(24)00781-1)
14. Ahern TP, Broe A, Lash TL, Cronin-Fenton DP, Ulrichsen SP, Christiansen PM, et al. Phthalate Exposure and Breast Cancer Incidence: A Danish Nationwide Cohort Study. *Journal of Clinical Oncology*. 2019;37(21):1800-9. DOI: <https://doi.org/10.1200/JCO.18.02202>
15. Olalekan Adeyeye SA, Ashaolu TJ. Heterocyclic Amine Formation and Mitigation in Processed Meat and Meat Products: A Mini-Review. *Journal of Food Protection*. 2021;84(11):1868-77. DOI: <https://doi.org/10.4315/JFP-21-936>
16. Jian S-H, Yeh P-J, Wang C-H, Chen H-C, Chen S-F. Analysis of heterocyclic amines in meat products by liquid chromatography – Tandem mass spectrometry. *Journal of Food and Drug Analysis*. 2019;27(2):595-602. DOI: <https://doi.org/10.1016/j.jfda.2018.12.006>
17. Choi SW, Mason JB. COLON | Cancer of the Colon. In: Caballero B, editor. *Encyclopedia of Food Sciences and Nutrition* (Second Edition). Oxford: Academic Press; 2003. p. 1543-50.
18. Rietjens I. Hazards and risks of process related contaminants in feed and foods of animal origin formed as a result of heating. 2019. p. 263-80.
19. Cascella M, Bimonte S, Barbieri A, Vecchio V, Caliendo D, Schiavone V, et al. Dissecting the mechanisms and molecules underlying the potential carcinogenicity of red and processed meat in colorectal cancer (CRC): An overview on the current state of knowledge. *Infectious Agents and Cancer*. 2018;13. DOI: <https://doi.org/10.1186/s13027-018-0174-9>

20. Moorthy B, Chu C, Carlin D. Polycyclic Aromatic Hydrocarbons: From Metabolism to Lung Cancer. *Toxicological sciences : an official journal of the Society of Toxicology*. 2015;145:5-15. DOI: <https://doi.org/10.1093/toxsci/kfv040>
21. Ejeomo C, Ogboje SU, Obayagbona NO, Ewansiha IC, Afure AM. Effect of Polynuclear Aromatic Hydrocarbons Levels in Suya and Barbecue Fish on Cancer Risk Index in Warri Metropolis, Southern Nigeria. *Journal of Agriculture, Food and Environment (JAFE)*. 2025;6(1):5-12.
22. Omoruyi IM, Ahamioje D, Pohjanvirta R. Dietary exposure of Nigerians to mutagens and estrogen-like chemicals. *Int J Environ Res Public Health*. 2014;11(8):8347-67. DOI: <https://doi.org/10.3390/ijerph110808347>
23. Edna Hee P-T, Liang Z, Zhang P, Fang Z. Formation mechanisms, detection methods and mitigation strategies of acrylamide, polycyclic aromatic hydrocarbons and heterocyclic amines in food products. *Food Control*. 2024;158:110236. DOI: <https://doi.org/10.1016/j.foodcont.2023.110236>
24. Adeyeye S. Heterocyclic Amines and Polycyclic Aromatic Hydrocarbons in Cooked Meat Products: A Review. *Polycyclic Aromatic Compounds*. 2018;40:1-11. DOI: <https://doi.org/10.1080/10406638.2018.1462828>
25. Oyewole OS, Balogun DA, Alao A-ZO, Ibitoye OS, Ajao TO, Ogungbemi K, et al. QUALITY ASSESSMENT AND SAFETY OF COMMERCIALY SOLD STEAK MEAT "SUYA" IN IBADAN METROPOLIS: A MENACE TO PUBLIC HEALTH. *FUDMA JOURNAL OF SCIENCES*. 2024;8(4):120 - 9.
26. Jang T-W, Kim Y, Won J-U, Lee J-S, Song J. The standards for recognition of occupational cancers related with polycyclic aromatic hydrocarbons (PAHs) in Korea. *Annals of Occupational and Environmental Medicine*. 2018;30(1):13. DOI: <https://doi.org/10.1186/s40557-018-0225-0>
27. Montano L, Baldini GM, Piscopo M, Liguori G, Lombardi R, Ricciardi M, et al. Polycyclic Aromatic Hydrocarbons (PAHs) in the Environment: Occupational Exposure, Health Risks and Fertility Implications. *Toxics*. 2025;13(3):151. DOI: <https://doi.org/10.3390/toxics13030151>
28. Ölmez M, Şahin T, Dağ S. Polycyclic Aromatic Hydrocarbons (PAHs) and Their Importance in Animal Nutrition. In: Kukovics S, editor. *Animal Husbandry*. Rijeka: IntechOpen; 2022.
29. Mallah MA, Changxing L, Mallah MA, Noreen S, Liu Y, Saeed M, et al. Polycyclic aromatic hydrocarbon and its effects on human health: An overview. *Chemosphere*. 2022;296:133948. DOI: <https://doi.org/10.1016/j.chemosphere.2022.133948>
30. Patel AB, Shaikh S, Jain KR, Desai C, Madamwar D. Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches. *Frontiers in Microbiology*. 2020;Volume 11 - 2020. DOI: <https://doi.org/10.3389/fmicb.2020.562813>
31. Balmer J, Hung H, Yu Y, Letcher R, Muir D. Sources and environmental fate of pyrogenic polycyclic aromatic hydrocarbons (PAHs) in the Arctic. *Emerging Contaminants*. 2019;5:128-42. DOI: <https://doi.org/10.1016/j.emcon.2019.04.002>
32. Fakolade PO, Ijiwade EO, Adeniyi AP. Polycyclic Aromatic Hydrocarbon (PAHs) and Phenols status in some smoked meat products in Nigeria. *Nigerian Journal of Animal Production*. 2017;44(4):118-27.
33. Sumer G, Oz F. The Effect of Direct and Indirect Barbecue Cooking on Polycyclic Aromatic Hydrocarbon Formation and Beef Quality. *Foods (Basel, Switzerland)*. 2023;12(7). DOI: <https://doi.org/10.3390/foods12071374>
34. Zastrow L, Judas M, Speer K, Schwind K-H, Jira W. Barbecue conditions affect contents of oxygenated and non-oxygenated polycyclic aromatic hydrocarbons in meat and non-meat patties. *Food Chemistry: X*. 2022;14:100351. DOI: <https://doi.org/10.1016/j.fochx.2022.100351>
35. Sumer G, Oz F. The Effect of Direct and Indirect Barbecue Cooking on Polycyclic Aromatic Hydrocarbon Formation and Beef Quality. *Foods*. 2023;12(7):1374. DOI: <https://doi.org/10.3390/foods12071374>
36. Rose M, Holland J, Dowding A, Petch R, White S, Fernandes A, et al. Investigation into the

- formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. *Food and Chemical Toxicology*. 2015;78. DOI: <https://doi.org/10.1016/j.fct.2015.02.019>
37. Giuliani A, Zuccarini M, Cichelli A, Khan H, Reale M. Critical Review on the Presence of Phthalates in Food and Evidence of Their Biological Impact. *Int J Environ Res Public Health*. 2020;17(16). DOI: <https://doi.org/10.3390/ijerph17165655>
 38. Pillo G, Aldrovandi F, Mescoli A, Maffei G, Mascolo MG, Vaccari M, et al. An insight into carcinogenic activity and molecular mechanisms of Bis(2-ethylhexyl) phthalate. *Frontiers in Toxicology*. 2024;Volume 6 - 2024. DOI: <https://doi.org/10.3389/ftox.2024.1358603>
 39. Wang Y-C, Chen H-S, Long C-Y, Tsai C-F, Hsieh T-H, Hsu C-Y, et al. Possible mechanism of phthalates-induced tumorigenesis. *The Kaohsiung Journal of Medical Sciences*. 2012;28(7, Supplement):S22-S7. DOI: <https://doi.org/10.1016/j.kjms.2012.05.002>
 40. Yang L, Liu X, Peng Z, Liu Z, Song P, Zhou J, et al. Exposure to di-2-ethylhexyl phthalate (DEHP) increases the risk of cancer. *BMC Public Health*. 2024;24(1):430. DOI: <https://doi.org/10.1186/s12889-024-17875-6>
 41. Arrigo F, Impellitteri F, Piccione G, Faggio C. Phthalates and their effects on human health: Focus on erythrocytes and the reproductive system. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2023;270:109645. DOI: <https://doi.org/10.1016/j.cbpc.2023.109645>
 42. Arukwe D, Okoli J, Chimezie UG. Functional, chemical and organoleptic properties of moi-moi prepared from blends of cowpea (*Vigna unguiculata*) and sprouted pigeon pea (*Cajanus cajan*) flours. *Science World Journal*. 2023;18:438-44.
 43. Iguh BN, Bede EN, Akanbi MN. Assessment and Characterization of Residual Concentrations from Some Cook-in Food Packages Utilized in Nigeria – ‘Moi-Moi’: A Case Study. *Asian Food Science Journal*. 2023;22(3):1-10.
 44. Bin-Jumah M, Abdel-Fattah AM, Saied EM, El-Seedi HR, Abdel-Daim MM. Acrylamide-induced peripheral neuropathy: manifestations, mechanisms, and potential treatment modalities. *Environ Sci Pollut Res Int*. 2021;28(11):13031-46. DOI: <https://doi.org/10.1007/s11356-020-12287-6>
 45. Mérida DM, Rey-García J, Moreno-Franco B, Guallar-Castillón P. Acrylamide Exposure and Cardiovascular Risk: A Systematic Review. *Nutrients*. 2024;16(24). DOI: <https://doi.org/10.3390/nu16244089>
 46. Li Y, Liu J, Wang Y, Wei S. Cancer risk and disease burden of dietary acrylamide exposure in China, 2016. *Ecotoxicology and Environmental Safety*. 2022;238:113551. DOI: <https://doi.org/10.1016/j.ecoenv.2022.113551>
 47. Al-Kindi S, Al-Bahry S, Al-Wahaibi Y, Taura U, Joshi S. Partially hydrolyzed polyacrylamide: enhanced oil recovery applications, oil-field produced water pollution, and possible solutions. *Environmental Monitoring and Assessment*. 2022;194. DOI: <https://doi.org/10.1007/s10661-022-10532-8>
 48. Eisenbrand G. Revisiting the evidence for genotoxicity of acrylamide (AA), key to risk assessment of dietary AA exposure. *Archives of Toxicology*. 2020;94(9):2939-50. DOI: <https://doi.org/10.1007/s00204-020-02834-y>
 49. Akinosun TO, Ojinnaka D, Aouzelleg A. The Effects of Different Cooking Conditions on the Level of Acrylamide in Popular West African Foods. *Scholarly Journal of Food and Nutrition*. 2025.
 50. Health ECDGf, Safety F. Trans-fats in food 2019 [updated 2019/04/24. Available from: https://food.ec.europa.eu/food-safety/labelling-and-nutrition/trans-fat-food_en#:~:text=Trans%20fats%20are%20a%20particular,trans%20fats%20are%20produced%20industrially.
 51. European C. Trans fat in food. European Commission - Food Safety. 2024.
 52. Dhaka V, Gulia N, Ahlawat KS, Khatkar BS. Trans fats-sources, health risks and alternative approach - A review. *J Food Sci Technol*. 2011;48(5):534-41. DOI: <https://doi.org/10.1007/s13197-010-0225-8>

53. Lingas EC. Early-Onset Colon Cancer: A Narrative Review of Its Pathogenesis, Clinical Presentation, Treatment, and Prognosis. *Cureus*. 2023;15(9):e45404. DOI: <https://doi.org/10.7759/cureus.45404>
54. Oteng A-B, Kersten S. Mechanisms of Action of trans Fatty Acids. *Advances in Nutrition*. 2020;11(3):697-708. DOI: <https://doi.org/10.1093/advances/nmaa012>
55. Addo P, Chen J, Bo Z, Li X, Zhang L, Tano-Debrah K. Trans Fatty Acids and Potential Factors that Influence Their Occurrence in Fried Foods: A Case Study on Plantain Chips Sold within the Accra Metropolis, Ghana. *SSRN Electronic Journal* 2023.
56. Khoshakhlagh AH, Mohammadzadeh M, Gruszecka-Kosowska A. The preventive and carcinogenic effect of metals on cancer: a systematic review. *BMC Public Health*. 2024;24(1):2079. DOI: <https://doi.org/10.1186/s12889-024-19579-7>
57. Mohammadi MJ, Kiani F, Farhadi M, Ghanbari S, Jalili D, Mirzaei L. Evaluation of carcinogenic risk of heavy metals due to consumption of rice in Southwestern Iran. *Toxicology Reports*. 2024;12:578-83. DOI: <https://doi.org/10.1016/j.toxrep.2024.05.004>
58. Kamalesh R, Saravanan A, Lyu P. Heavy metal exposure as a risk factor in oral cancer. *Cancer Pathogenesis and Therapy*. 2024;02(03):215-6. DOI: <https://doi.org/10.1016/j.cpt.2024.04.003>
59. Caprara G, Pallavi R, Sanyal S, Pelicci PG. Dietary Restrictions and Cancer Prevention: State of the Art. *Nutrients*. 2025;17(3):503. DOI: <https://doi.org/10.3390/nu17030503>
60. Dominguez LJ, Veronese N, Di Bella G, Cusumano C, Parisi A, Tagliaferri F, et al. Mediterranean diet in the management and prevention of obesity. *Experimental Gerontology*. 2023;174:112121. DOI: <https://doi.org/10.1016/j.exger.2023.112121>
61. Khalifa A, Guijarro A, Nencioni A. Advances in Diet and Physical Activity in Breast Cancer Prevention and Treatment. *Nutrients*. 2024;16(14). DOI: <https://doi.org/10.3390/nu16142244>
62. Kerschbaum E, Nüssler V. Cancer Prevention with Nutrition and Lifestyle. *Visc Med*. 2019;35(4):204-9. DOI: <https://doi.org/10.1159/000501776>
63. Kumari P, Gaur SS, Tiwari RK. Banana and its by-products: A comprehensive review on its nutritional composition and pharmacological benefits. *eFood*. 2023;4(5):e110. DOI: <https://doi.org/10.1002/efd2.110>
64. Lau BF, Kong KW, Leong KH, Sun J, He X, Wang Z, et al. Banana inflorescence: Its bio-prospects as an ingredient for functional foods. *Trends in Food Science & Technology*. 2020;97:14-28. DOI: <https://doi.org/10.1016/j.tifs.2019.12.023>
65. Bangar SP, Dunno K, Dhull SB, Kumar Siroha A, Changan S, Maqsood S, et al. Avocado seed discoveries: Chemical composition, biological properties, and industrial food applications. *Food Chemistry: X*. 2022;16:100507. DOI: <https://doi.org/10.1016/j.fochx.2022.100507>
66. Dayı T, Özsoy S, Bozkurt AY. Avocado (*Persea americana*) and Potential Anticancer Effects: Do the Effects Suppress Carcinogenesis? *Cyprus Journal of Medical Sciences*. 2025;10(1):1-6.
67. Farghadani R, Naidu R. The anticancer mechanism of action of selected polyphenols in triple-negative breast cancer (TNBC). *Biomedicine & Pharmacotherapy*. 2023;165:115170. DOI: <https://doi.org/10.1016/j.biopha.2023.115170>
68. Chen LC, Chang HS, Ho YS. A deep dive into the orchard of health: Exploring the anti-cancer and anti-aging potential of apple polyphenols. *J Food Drug Anal*. 2025;33(1):1-12. DOI: <https://doi.org/10.38212/2224-6614.3508>
69. Nezbedova L, McGhie T, Christensen M, Heyes J, Nasef NA, Mehta S. Onco-Preventive and Chemo-Protective Effects of Apple Bioactive Compounds. *Nutrients*. 2021;13(11). DOI: <https://doi.org/10.3390/nu13114025>
70. Lima NNdC, Faustino DC, Allahdadi KJ, França LSdA, Pinto LC. Acetogenins from Annonaceae plants: potent antitumor and neurotoxic compounds. *PharmaNutrition*. 2022;20:100295.

- DOI: <https://doi.org/10.1016/j.phanu.2022.100295>
71. Bhadra K. Anticancer Natural Alkaloids as Drug Bank Targeting Biomolecules. In: Hussain CM, Di Sia P, editors. Handbook of Smart Materials, Technologies, and Devices: Applications of Industry 40. Cham: Springer International Publishing; 2022. p. 559-89.
72. Siddiqui SA, Singh S, Nayik GA. Bioactive compounds from pomegranate peels - Biological properties, structure-function relationships, health benefits and food applications – A comprehensive review. Journal of Functional Foods. 2024;116:106132. DOI: <https://doi.org/10.1016/j.jff.2024.106132>
73. Rauf A, Olatunde A, Akram Z, Hemeg HA, Aljohani ASM, Al Abdulmonem W, et al. The Role of Pomegranate (*Punica granatum*) in Cancer Prevention and Treatment: Modulating Signaling Pathways From Inflammation to Metastasis. Food Science & Nutrition. 2025;13(2):e4674. DOI: <https://doi.org/10.1002/fsn3.4674>
74. Golmohammadi M, Zamanian MY, Jalal SM, Noraldein SAM, Ramírez-Coronel AA, Oudaha KH, et al. A comprehensive review on Ellagic acid in breast cancer treatment: From cellular effects to molecular mechanisms of action. Food Science & Nutrition. 2023;11(12):7458-68. DOI: <https://doi.org/10.1002/fsn3.3728>
75. Milella RA, De Rosso M, Gasparro M, Gigante I, Debiase G, Forleo LR, et al. Correlation between antioxidant and anticancer activity and phenolic profile of new Apulian table grape genotypes (*V. Vinifera* L.). Frontiers in Plant Science. 2023;Volume 13 - 2022. DOI: <https://doi.org/10.3389/fpls.2022.1095545>
76. Quero J, Jiménez-Moreno N, Esparza I, Osada J, Cerrada E, Ancín-Azpilicueta C, et al. Grape Stem Extracts with Potential Anticancer and Antioxidant Properties. Antioxidants. 2021;10(2):243. DOI: <https://doi.org/10.3390/antiox10020243>
77. Ferraz da Costa DC, Pereira Rangel L, Quarti J, Santos RA, Silva JL, Fialho E. Bioactive Compounds and Metabolites from Grapes and Red Wine in Breast Cancer Chemoprevention and Therapy. Molecules. 2020;25(15):3531. DOI: <https://doi.org/10.3390/molecules25153531>
78. Zhou K, Raffoul JJ. Potential anticancer properties of grape antioxidants. J Oncol. 2012;2012:803294. DOI: <https://doi.org/10.1155/2012/803294>
79. Zhou DD, Li J, Xiong RG, Saimaiti A, Huang SY, Wu SX, et al. Bioactive Compounds, Health Benefits and Food Applications of Grape. Foods. 2022;11(18). DOI: <https://doi.org/10.3390/foods11182755>
80. Stabrauskiene J, Kopustinskiene DM, Lazauskas R, Bernatoniene J. Naringin and Naringenin: Their Mechanisms of Action and the Potential Anticancer Activities. Biomedicines. 2022;10(7). DOI: <https://doi.org/10.3390/biomedicines10071686>
81. Tajodini M, Samadi F, Asadi J, Khosravi A, Samadi F. Anticancer and apoptotic effects of orange peel extract and naringin on doxorubicin-induced apoptosis in human esophageal squamous carcinoma cell line 2023.
82. Tajaldini M, Samadi F, Khosravi A, Ghasemnejad A, Asadi J. Protective and anticancer effects of orange peel extract and naringin in doxorubicin treated esophageal cancer stem cell xenograft tumor mouse model. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020;121:109594. DOI: <https://doi.org/10.1016/j.biopha.2019.109594>
83. de Castro DdSB, Teodoro AJ. Anticancer Properties of Bioactive Compounds of Berry Fruits - A Review. Journal of Advances in Medicine and Medical Research. 2015;6(8):771-94.
84. Alsharairi NA. A Review with a Focus on Vaccinium-Berries-Derived Bioactive Compounds for the Treatment of Reproductive Cancers. Plants (Basel). 2024;13(7). DOI: <https://doi.org/10.3390/plants13070958>
85. Ağagündüz D, Şahin T, Yılmaz B, Ekenci KD, Duyar Özer Ş, Capasso R. Cruciferous Vegetables and Their Bioactive Metabolites: from Prevention to Novel Therapies of Colorectal Cancer. Evid Based Complement Alternat Med. 2022;2022:1534083. DOI: <https://doi.org/10.1155/2022/1534083>

86. Motlhalamme T, Kaningini AG, Maropeng R, Thema FT, Mohale KC, Maaza M. Tomato lycopene, total flavonoids, vitamin C content and mineral composition as affected by biosynthesized calcium carbonate nanoparticles foliar application. *Journal of Food Composition and Analysis*. 2025;139:107173.
DOI: <https://doi.org/10.1016/j.jfca.2025.107173>
87. Ozkan G, Günal-Köroğlu D, Karadag A, Capanoglu E, Cardoso SM, Al-Omari B, et al. A mechanistic updated overview on lycopene as potential anticancer agent. *Biomedicine & Pharmacotherapy*. 2023;161:114428.
DOI: <https://doi.org/10.1016/j.biopha.2023.114428>
88. Catanzaro E, Canistro D, Pellicioni V, Vivarelli F, Fimognari C. Anticancer potential of allicin: A review. *Pharmacological Research*. 2022;177:106106.
DOI: <https://doi.org/10.1016/j.phrs.2022.106106>
89. El-Saadony MT, Saad AM, Korma SA, Salem HM, Abd El-Mageed TA, Alkafaas SS, et al. Garlic bioactive substances and their therapeutic applications for improving human health: a comprehensive review. *Frontiers in Immunology*. 2024;Volume 15 - 2024.
DOI: <https://doi.org/10.3389/fimmu.2024.1356939>
90. Mondal A, Banerjee S, Bose S, Mazumder S, Haber RA, Farzaei MH, et al. Garlic constituents for cancer prevention and therapy: From phytochemistry to novel formulations. *Pharmacological Research*. 2022;175:105837.
DOI: <https://doi.org/10.1016/j.phrs.2021.105837>
91. Li Z, Le W, Cui Z. A novel therapeutic anticancer property of raw garlic extract via injection but not ingestion. *Cell Death Discovery*. 2018;4(1):108.
DOI: <https://doi.org/10.1038/s41420-018-0122-x>
92. Zhang Y, Liu X, Ruan J, Zhuang X, Zhang X, Li Z. Phytochemicals of garlic: Promising candidates for cancer therapy. *Biomedicine & Pharmacotherapy*. 2020;123:109730.
DOI: <https://doi.org/10.1016/j.biopha.2019.109730>
93. Talib WH, Atawneh S, Shakhathreh AN, Shakhathreh GaN, Rasheed aljarrah IS, Hamed RA, et al. Anticancer potential of garlic bioactive constituents: Allicin, Z-ajoene, and organosulfur compounds. *Pharmacia*. 2024;71:1-23.
DOI: <https://doi.org/10.3897/pharmacia.71.e116292>
94. Mandrich L, Esposito AV, Costa S, Caputo E. Chemical Composition, Functional and Anticancer Properties of Carrot. *Molecules*. 2023;28(20).
DOI: <https://doi.org/10.3390/molecules28207041>
95. Park HY, Lee S, Kim SH, Park JE, Hwang YS, Kang MH, et al. Anticancer effects of purple carrot extract via induction of apoptotic genes on human breast cancer cells. *Food Science and Biotechnology*. 2025;34(8):1737-49.
DOI: <https://doi.org/10.1007/s10068-024-01738-0>
96. Gupta A, Atanasov AG, Li Y, Kumar N, Bishayee A. Chlorogenic acid for cancer prevention and therapy: Current status on efficacy and mechanisms of action. *Pharmacol Res*. 2022;186:106505.
DOI: <https://doi.org/10.1016/j.phrs.2022.106505>
97. Yasin M, Li L, Mak M, Chen Z-H, Panchal S. Capsicum Waste as a Sustainable Source of Capsaicinoids for Metabolic Diseases. *Foods*. 2023;12:907.
DOI: <https://doi.org/10.3390/foods12050907>
98. Hudáková T, Šemeláková M, Očenáš P, Kožurková M, Krochtová K, Sovová S, et al. Chili pepper extracts, capsaicin, and dihydrocapsaicin as potential anticancer agents targeting topoisomerases. *BMC Complementary Medicine and Therapies*. 2024;24(1):96.
DOI: <https://doi.org/10.1186/s12906-024-04395-4>
99. Tomeh MA, Hadianamrei R, Zhao X. A Review of Curcumin and Its Derivatives as Anticancer Agents. *International journal of molecular sciences*. 2019;20(5).
DOI: <https://doi.org/10.3390/ijms20051033>
100. Cozmin M, Lungu, II, Gutu C, Stefanache A, Duceac LD, Şoltuzu BD, et al. Turmeric: from spice to cure. A review of the anti-cancer, radioprotective and anti-inflammatory effects of turmeric sourced compounds. *Front Nutr*. 2024;11:1399888.
DOI: <https://doi.org/10.3389/fnut.2024.1399888>

101. Cozmin M, Lungu II, Gutu C, Stefanache A, Duceac LD, Şoltuzu BD, et al. Turmeric: from spice to cure. A review of the anti-cancer, radioprotective and anti-inflammatory effects of turmeric sourced compounds. *Frontiers in Nutrition*. 2024;Volume 11 - 2024. DOI: <https://doi.org/10.3389/fnut.2024.1399888>
102. Zhang X-F, Wang X-K, Tang Y-J, Guan X-X, Guo Y, Fan J-M, et al. Association of whole grains intake and the risk of digestive tract cancer: a systematic review and meta-analysis. *Nutrition Journal*. 2020;19(1):52. DOI: <https://doi.org/10.1186/s12937-020-00572-6>
103. Xie M, Liu J, Tsao R, Wang Z, Sun B, Wang J. Whole Grain Consumption for the Prevention and Treatment of Breast Cancer. *Nutrients*. 2019;11(8). DOI: <https://doi.org/10.3390/nu11081769>
104. Campos-Vega R, Oomah BD, Loarca-Piña G, Vergara-Castañeda HA. Common Beans and Their Non-Digestible Fraction: Cancer Inhibitory Activity-An Overview. *Foods*. 2013;2(3):374-92. DOI: <https://doi.org/10.3390/foods2030374>
105. Serventi L, Cai X, Chen R, Dilrukshi N, Su J, Tuange RPN, et al. Anticancer Properties of Aqueous Extracts from Leguminosae. *Nutraceuticals*. 2022;2(4):323-34. DOI: <https://doi.org/10.3390/nutraceuticals2040025>
106. Bolling BW, Aune D, Noh H, Petersen KS, Freisling H. Dried Fruits, Nuts, and Cancer Risk and Survival: A Review of the Evidence and Future Research Directions. *Nutrients*. 2023;15(6). DOI: <https://doi.org/10.3390/nu15061323>
107. Sun H, Yu W, Li H, Hu X, Wang X. Bioactive Components of Areca Nut: An Overview of Their Positive Impacts Targeting Different Organs. *Nutrients*. 2024;16(5). DOI: <https://doi.org/10.3390/nu16050661>
108. Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attri DC, et al. Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. *Cancer Cell International*. 2022;22(1):206. DOI: <https://doi.org/10.1186/s12935-022-02624-9>
109. Markellos C, Ourailidou ME, Gavriatopoulou M, Halvatsiotis P, Sergentanis TN, Psaltopoulou T. Olive oil intake and cancer risk: A systematic review and meta-analysis. *PLoS One*. 2022;17(1):e0261649. DOI: <https://doi.org/10.1371/journal.pone.0261649>
110. Rocchetti G, Luisa Callegari M, Senizza A, Giuberti G, Ruzzolini J, Romani A, et al. Oleuropein from olive leaf extracts and extra-virgin olive oil provides distinctive phenolic profiles and modulation of microbiota in the large intestine. *Food Chemistry*. 2022;380:132187. DOI: <https://doi.org/10.1016/j.foodchem.2022.132187>
111. Bounegru AV, Apetrei C. Studies on the Detection of Oleuropein from Extra Virgin Olive Oils Using Enzymatic Biosensors. *International journal of molecular sciences*. 2022;23(20):12569. DOI: <https://doi.org/10.3390/ijms232012569>
112. Rishmawi S, Haddad F, Dokmak G, Karaman R. A Comprehensive Review on the Anti-Cancer Effects of Oleuropein. *Life (Basel)*. 2022;12(8). DOI: <https://doi.org/10.3390/life12081140>

Review Article

Beyond Inhibition: Emerging Small-Molecule Modalities in Oncology (Molecular Glues, Covalents, and Radiotheranostics)

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Abstract:

Background: Small-molecule drugs have transformed oncology, but conventional inhibitors are limited by resistance, restricted target scope, and declining durability.

Methods: We reviewed emerging small-molecule modalities—molecular glues, covalent inhibitors, and radiotheranostics—focusing on their mechanisms, clinical applications, and translational challenges. Key clinical trials and representative examples were identified from recent oncology literature.

Results: Molecular glues enable targeted degradation of previously undruggable proteins, with clinical success in multiple myeloma (IMiDs). Covalent inhibitors achieve durable suppression of oncogenic drivers such as KRAS^{G12C} and BTK, as shown in CodeBreak100 (sotorasib; N=126; ORR 37%). Radiotheranostics combine imaging and therapy, exemplified by VISION (PSMA-617; N=831; OS HR 0.62) and NETTER-1 (Lutathera; N=229; PFS HR 0.21). Collectively, these modalities expand the druggable proteome, improve durability, and advance precision oncology.

Conclusion: Emerging small-molecule approaches mark a paradigm shift from inhibition alone to targeted degradation, durable covalent engagement, and diagnostic–therapeutic hybrids. Future priorities include improving selectivity, biomarker integration, scalable manufacturing, and equitable global access.

Keywords: Molecular Glues; Covalent Inhibitors; Radiotheranostics; Oncology Drug Discovery; Targeted Protein Degradation; Precision Oncology

Introduction

For the last forty years, tiny molecules have been very important to making progress in cancer treatment. From the early use of hormone therapies for breast and prostate cancer to the introduction of kinase inhibitors that changed the way chronic myeloid leukaemia and non-small cell lung cancer are treated, these drugs have shown that chemical precision can lead to big improvements in health (1). The path of traditional small-molecule drug discovery has, however, reached a crucial turning point. Resistance to inhibitors arises almost invariably, whether via secondary mutations in the drug target, adaptive bypass routes, or modifications in drug transport and metabolism. Also, the fact that humans rely on well-defined catalytic niches has made many parts of the cancer proteome, especially transcription factors, scaffolding proteins, and disordered domains, "undruggable"(2). These facts show both the strengths and weaknesses of the classic inhibition model.

Recent advancements have expanded the chemical conceptualisation in oncology beyond the mere inhibition of enzyme activity. A new generation of small-molecule methods is pushing the limits of what is possible by using alternative ways to regulate proteins and deliver drugs. Molecular glues, for example, do not stop new protein-protein connections; instead, they encourage them. This changes the way cells break down proteins, making it easier for oncogenic drivers to get to

them. Covalent small compounds were originally looked at with trepidation because of safety concerns, but today they are known to be able to permanently silence hard-to-reach targets like KRAS^{G12C} and BTK. Simultaneously, radiotheranostics are changing the role of tiny molecules into precise tools that can diagnose and cure cancer at the same time through isotope conjugation (3).

This study analyses these three new modalities, focusing on their molecular underpinnings, clinical uses, and translational challenges. We want to show both their potential and the important questions that will influence their future by putting them in the context of the larger history of small-molecule oncology.

Despite decades of progress, the field now faces a critical challenge: conventional small-molecule inhibitors are increasingly limited by resistance, restricted target scope, and diminishing clinical returns. Addressing these limitations is significant because most oncogenic drivers remain "undruggable," and patients with resistant disease urgently need more durable therapeutic options. This study examines three emerging small-molecule modalities—molecular glues, covalent inhibitors, and radiotheranostics—as innovative strategies to overcome these barriers and redefine the future of precision oncology.

Limitations of Conventional Small-Molecule Inhibitors

The literature used in this review was selected from peer-reviewed articles published between 2015 and 2020 from databases such as PubMed, Scopus and Google Scholar using search terms such as 'drug discovery', 'pharmacological innovations', 'AI in drug development', 'challenges of drug discovery'. Papers were selected based on relevance to the theme, content quality and recency. No protocol of systematic review was used. Generative AI tools were not used in the preparation of this manuscript. Minor language editing tools were used to improve clarity and grammar.

Even while they have the potential to change things, traditional small-molecule inhibitors have well-known problems that restrict their usefulness in the long term. One of the most important of these is the fact that resistance is almost certain to happen. Tumour cells are very good at changing the way they send signals to avoid drug pressure. For tyrosine kinase inhibitors, subsequent mutations in the target often make it harder for the medicine to bind. For example, the EGFR T790M mutation in lung cancer or the G1202R mutation in ALK-driven tumours (4).

Even when the main binding site is still there, malignancies often use adaptive bypass signalling to turn on parallel pathways that bring back signals for growth and survival. Efflux pumps and changes in metabolic pathways make it even harder for drugs to build up inside cells. This makes it possible for tumours that were sensitive at first to come back. The range of targets available for classical inhibition is equally limited. Successful inhibitors usually depend on clearly defined, deep binding pockets that can hold a lot of affinity (5). This condition leaves out a lot of the proteome, including transcription factors, scaffolding proteins, and intrinsically disordered regions, which don't have such voids. These types of proteins are very important in the development of cancer, but they can't be targeted by typical drug design. This supports the idea of the "undruggable" proteome.

Selectivity is another problem that keeps coming up. Even when inhibitors have a high target affinity, it's hard to avoid off-target interactions, especially in protein families with conserved structural features, like kinases. This can lead to unforeseen physiological consequences, varying from mild side

effects to dose-limiting toxicities. At the same time, great on-target potency may not necessarily be a good thing. For example, some kinase inhibitors might be cardiotoxic because they completely block signalling, which stops normal tissue activities (6).

All of these things together make up what could be called a clinical plateau. Inhibitors often produce remarkable initial responses, especially in genetically characterised malignancies; nevertheless, these advantages are typically ephemeral. Resistance develops, tumour heterogeneity manifests, and lasting cures continue to be unattainable for the majority of advanced malignancies. Consequently, the field faces a biological limitation intrinsic to the paradigm of

inhibition. To go over this plateau, we need treatment techniques that can extend the druggable proteome, get around adaptive resistance, and keep tumours under control for a long time without causing too much harm. These unmet needs have spurred the quest for novel small-molecule modalities that transcend mere inhibition to embrace fundamentally distinct mechanisms of action (7).

These unmet needs have spurred the quest for novel small-molecule modalities that transcend inhibition. The next section explores molecular glues, a pioneering approach that expands the druggable proteome through targeted degradation.

Molecular Glues: Inducing Protein-Protein Interactions

Mechanistic Basis

Molecular glues signify a transformative advancement in drug development, operating not as inhibitors but as enhancers of novel protein-protein interactions. Molecular glues don't stop an enzyme from working by blocking its active site. Instead, they stabilise weak or temporary connections between a target protein and an E3 ubiquitin ligase, which eventually leads to the target protein being broken down by the proteasomal pathway. This technique makes it possible to selectively get rid of proteins that have long been thought to be "undruggable," such as several transcription factors and scaffolding proteins that are important for cancer growth (8). Figure 1 shows how molecular glues work at their most basic level. It

depicts how the glue molecule brings the E3 ligase and the target protein closer together, which helps the ubiquitination process. This leads to the target being broken down by proteasomes, which stops it from being able to cause cancer. This change from stopping proteins from working to breaking them down is a new way to treat cancer. It opens up new proteins that were previously inaccessible to classic small-molecule therapies (3). Table 1 gives a summary of the differences across small-molecule modalities, including their mechanisms, clinical uses, and examples. This table makes it easy to see how Molecular Glues, Covalent Inhibitors, and Radiotheranostics all work differently to fight cancer.

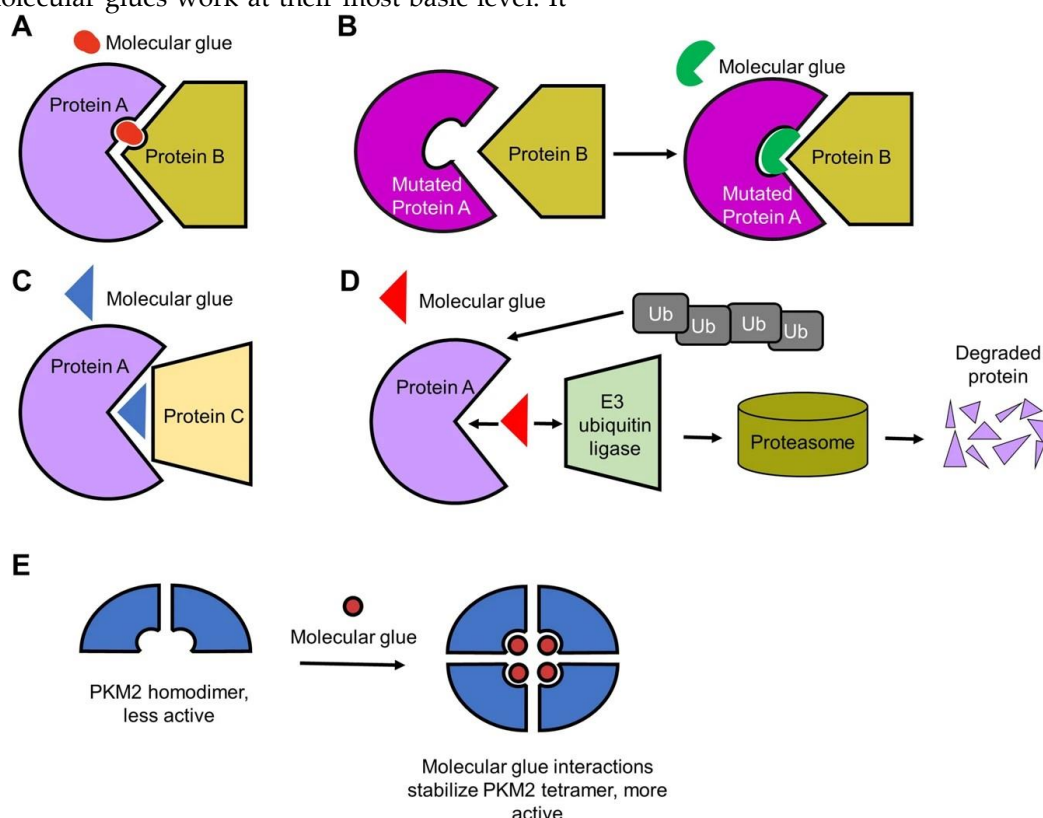


Figure 1 (Molecular Glue Mechanism).

“Mechanism of Molecular Glue-Mediated Targeted Protein Degradation. The schematic shows how molecular glues bridge an E3 ligase and a protein of interest (POI), leading to polyubiquitination and proteasomal degradation. Unlike inhibitors that block function, glues catalytically eliminate the protein, expanding druggable targets.” Weigel EG, Foulks JM,

Siddiqui A, Warner SL. Molecular glues: enhanced protein-protein interactions and cell proteome editing.

Table 1 (Comparison of Modalities): Comparison of Small-Molecule Modalities. The table outlines the mechanisms, representative clinical applications, and examples of molecular glues, covalent inhibitors, and radiotheranostics.

Modality	Mechanism	Clinical Applications	Examples
Molecular Glues(8)	Facilitate protein-protein interactions, leading to protein degradation	Targeting transcription factors and scaffolding proteins in cancer	Thalidomide, Lenalidomide
Covalent Inhibitors(9)	Irreversible bond formation with target proteins	Inhibition of oncogenic drivers like KRAS, BTK	Sotorasib (KRAS G12C), Ibrutinib (BTK)
Radiotheranostics(10)	Conjugation of small molecules to radioisotopes for imaging and therapy	Diagnosis and treatment of cancer, e.g., prostate cancer	PSMA-617 (prostate cancer), Lutetium-177-DOTATATE (neuroendocrine tumors)

Historical Perspective

The field's origins are closely linked to thalidomide, which was first sold as a sedative in the 1950s. Its retraction due to disastrous teratogenic consequences serves as a cautionary example in pharmacology. But decades later, the discovery that thalidomide and its derivatives work as molecular glues for the CRBN E3 ligase complex changed its legacy. Thalidomide drugs, lenalidomide and pomalidomide, became powerful immunomodulatory medicines (IMiDs) by encouraging the breakdown of important transcription factors in multiple myeloma. This dual legacy shows both the risks of unexpected off-target interactions and the chances that come with rethinking molecular mechanisms. This sets the foundation for the systematic creation of glue-based degraders (11).

Key Case Studies

The IMiD class exemplifies molecular glues. Lenalidomide and pomalidomide have become pivotal in the treatment of multiple myeloma by enlisting the E3 ligase cereblon to facilitate the degradation of transcription factors like IKAROS Family Zinc Finger 1 (IKZF1) and IKAROS Family Zinc Finger 3 (IKZF3). Their success confirmed that degradation, rather than only inhibition, could ensure lasting disease management. Significantly, these drugs demonstrated the potential immunomodulatory effects of targeted degradation, as their anticancer activity is partially derived from modified cytokine signalling and T cell activation (12).

Indisulam has recently shown that molecular glue action is not limited to cereblon. Indisulam facilitates the recruitment of the DCAF15 substrate receptor, resulting in the degradation of RBM39, an RNA splicing factor. Despite its inconsistent clinical trajectory, indisulam demonstrated that novel ligase-substrate combinations may be utilised by relatively tiny compounds. This facilitated the expansion of glue-mediated degradation.

In addition to Cereblon (CRBN) and DCAF15, current research has uncovered new degraders that function via different ligases or unique substrate profiles. This includes chemicals that function independently of CRBN, expanding the resources for addressing resistant tumours and diversifying the degradable proteome (13). Collectively, these case studies highlight that molecular glues can reveal therapeutic vulnerabilities that orthodox drug discovery methods have overlooked.

Oncologic Applications

Molecular glues hold a lot of promise as medicines, notably in cancer treatment, where transcription factors and scaffolding proteins are very important yet have always been hard to work with in traditional pharmacology. IKZF1 and IKZF3 degradation in multiple myeloma highlights this accomplishment, indicating that transcriptional regulators can be successfully removed in a therapeutic environment (14). Emerging glue degraders that target splicing factors and scaffolding proteins also point to a way to break down cancer-causing networks that rely

on protein–protein interactions instead of enzymes. These applications highlight the distinctive capacity of molecular glues to convert hitherto unattainable proteins into viable therapeutic targets in haematologic and potentially solid cancers (8).

Challenges

Molecular glues are quite difficult to work with, even though they hold a lot of promise. It is still not easy to predict degradable substrates because interactions caused by glue depend on small structural compatibilities. Resistance can develop due to mutations in E3 ligase components, as evidenced in clinical contexts. These problems show how important it is to have better discovery platforms and a better understanding of how glue-mediated relationships work (15).

Future Outlook

Rational and computationally guided discovery is the way forward for molecular glues. Artificial intelligence and machine learning are starting to be able to forecast how well ligases and substrates will work together. This opens up new methods to create things instead of just finding them by chance. To fully realise their therapeutic potential, it will be necessary to extend beyond CRBN and VHL to a more extensive ligase repertoire. Table 2 shows that each new modality has its own pros and cons. This table shows the pros and cons of each modality for use in oncology, including target range, durability, and safety. It also lists the problems that still need to be fixed (16).

While molecular glues open new doors for targeting undruggable proteins, another emerging class—covalent inhibitors—demonstrates how chemical precision can be leveraged to achieve durable target engagement.

Table 2. Advantages and Challenges of Emerging Modalities:

Modality	Advantages	Challenges
Molecular Glues(16)	Target previously 'undruggable' proteins, with potential for durable response	Predicting degradable substrates, resistance through ligase mutations
Covalent Inhibitors(9)	Sustained target inhibition, overcoming resistance, high potency	Off-target reactivity, potential immunogenicity, and irreversible binding risks
Radiotheranostics(10)	Dual function: imaging and therapy, precise tumor targeting	Challenges in isotope production, off-target accumulation, and limited tumor accessibility

Covalent Small Molecules: Locking Targets with Chemical Precision

Mechanistic Rationale

Covalent inhibitors are a conscious revival of one of chemistry's oldest techniques: utilising bonds that can't be broken to stop biological action. Covalent drugs, on the other hand, create a persistent chemical bond—usually to a nucleophilic residue like cysteine, lysine, or serine—within the active site or a nearby regulatory domain. This is different from typical reversible inhibitors, which depend on equilibrium occupancy. This link guarantees extended target inactivation, surpassing the plasma clearance of the medication and thereby separating pharmacokinetics from pharmacodynamics. This method works

especially well for kinases and other proteins that have cysteine residues that are easy to get to near functional regions (17).

By covalently attaching the inhibitor, it is possible to get a high level of activity even when the systemic concentrations are low. Additionally, these connections can avoid competition with plentiful cellular substrates like ATP. This mechanical rationale is what has brought back interest in covalent small molecules in oncology. What was formerly seen as risky is now a key part of therapeutic discovery (18). Figure 1 shows how covalent inhibitors make permanent connections with their target proteins, which keeps them from working.

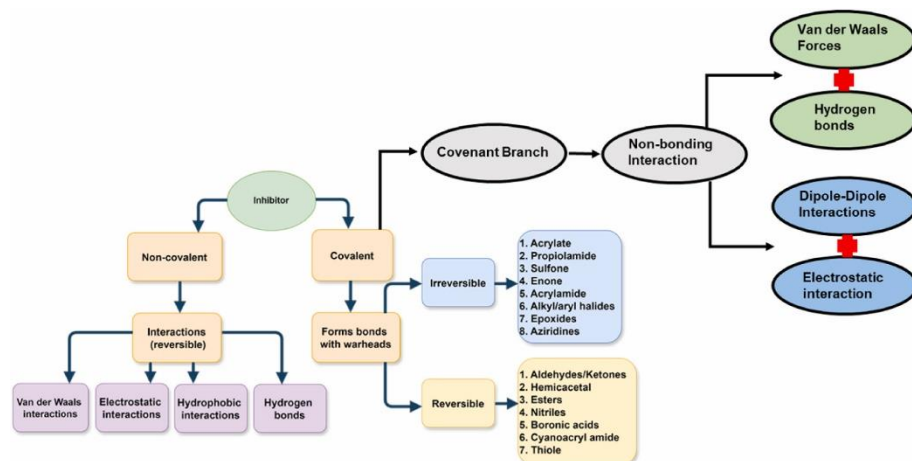


Figure [1]: Mechanism of Small-Molecule Inhibition via Covalent Bond Formation.

This figure illustrates the interaction between a covalent inhibitor and its target protein, highlighting the formation of a covalent bond between the inhibitor and a nucleophilic residue in the protein's active site. The diagram emphasizes the irreversible nature of this binding, which leads to prolonged target inhibition and can overcome certain resistance mechanisms associated with reversible inhibitors. This mechanism is exemplified by covalent inhibitors targeting proteins like KRAS^{G12C} and Bruton's tyrosine kinase (BTK), showcasing their potential in oncology therapeutics.

Case Studies in Oncology

The most obvious triumph of covalent chemistry in cancer treatment is the creation of KRAS^{G12C} inhibitors. For many years, KRAS was a symbol of the "undruggable" oncogene. The identification of a reactive cysteine at codon 12 facilitated the development of drugs such as sotorasib (AMG 510) and adagrasib. These medications permanently change KRAS^{G12C}, keeping it in an inactive GDP-bound state and stopping MAPK signalling from happening. Clinical trials have shown significant responses in non-small cell lung cancer, representing a significant achievement in addressing a long-sought driver (19).

By targeting Bruton's tyrosine kinase (BTK), covalent inhibitors have also changed the way haematologic cancers are treated. Ibrutinib, the first drug in its class, makes a covalent link with Cys481, which stops B-cell receptor signalling. Later drugs like acalabrutinib and zanubrutinib improved this method by making it more selective, which lowered off-target toxicities while keeping BTK inhibition strong (20).

Covalent inhibition has improved the treatment of EGFR-mutant lung cancer in solid tumours. Osimertinib, an irreversible EGFR inhibitor, was especially engineered to counteract the T790M resistance mutation that compromised previous generations of reversible inhibitors. Osimertinib exerts

strong suppression by covalently binding to Cys797 at the ATP-binding region. This leads to clinically significant increases in progression-free survival (21). These case studies collectively demonstrate the adaptability of covalent methodologies across various oncogenic drivers.

Advantages

The pharmacological stability of covalent inhibitors is what makes them appealing. Because binding is permanent, target inhibition can last long after the medication has been cleared from the body. This means that smaller doses can be given more often, which may help keep drug levels more stable. This extended interaction is especially advantageous when contending with elevated amounts of natural substrates, such as ATP in kinase active sites. Covalent inhibitors can also get around some resistance mechanisms. For example, changes that make it harder for reversible ligands to bind may nevertheless leave reactive residues open for covalent capture. The result is a therapeutic profile that can include strength, selectivity, and clinical resilience (17).

Concerns

Even though covalent inhibitors have these benefits, they also have their own hazards. Off-target reactivity is still a big worry since random covalent binding could cause immunological responses or cell death. The interaction is permanent, which means that accidental changes to proteins could have long-lasting negative repercussions. Additionally, covalent binding can generate neoantigens that provoke immunogenicity, hence complicating safety evaluations. In oncology, where patients frequently undergo prolonged pharmacotherapy, these risks are significant. Resistance can also develop by mutations that remove or protect the reactive residue, as seen with specific BTK mutations. These risks make it even more important to plan carefully and keep a close eye on patients (22).

Emerging Innovations

The next generation of covalent medicines intends to develop this method with greater precision. Reversible covalent inhibitors, which generate bonds that can break down in the body, try to find a compromise between safety and durability. Improvements in covalent fragment-based drug discovery are broadening the range of chemicals that may be studied, making it possible to systematically look for reactive warheads and binding motifs. Covalent techniques are no longer limited to enzymes

and kinases; they are now being applied to non-enzymatic proteins, such as transcription factors and scaffolding proteins that were previously considered unattainable. These new ideas show that covalent chemistry will continue to be an important part of modern oncology, always changing to deal with problems of safety and resistance (23). Beyond covalent strategies, radiotheranostics illustrate how small molecules can serve as dual-purpose agents, bridging diagnosis and therapy in real time."

Radiotheranostics: Small Molecules as Diagnostic–Therapeutic Hybrids

Conceptual Framework

Radiotheranostics is a very promising new area of precision oncology. It combines the separate tasks of diagnosis and therapy into one chemical entity. The basic idea is simple: tiny compounds are made to only bind to tumor-associated antigens or molecular markers, and these ligands are linked to radioisotopes. The conjugate can either send diagnostic signals (usually by positron emission tomography) or send lethal radiation directly to cancerous cells, depending on the isotope chosen. This duality enables the utilisation of the same molecular scaffold for staging, therapy selection, and therapeutic intervention, establishing an unparalleled continuity of care(24).

Radiotheranostics are important because they combine real-time functional imaging with targeted therapy, which lets doctors measure how much of a tumour is taken up before treatment and adjust the dose accordingly. Radiotheranostics represent a paradigm change, converting tiny compounds into multifunctional diagnostic-therapeutic hybrids capable of directing precision treatment regimens with remarkable accuracy(10). Figure 1 shows how the radiotheranostic workflow works. It shows how tiny compounds attached to radioisotopes can help with both diagnostic imaging and targeted radiation therapy for tumour cells at the same time.

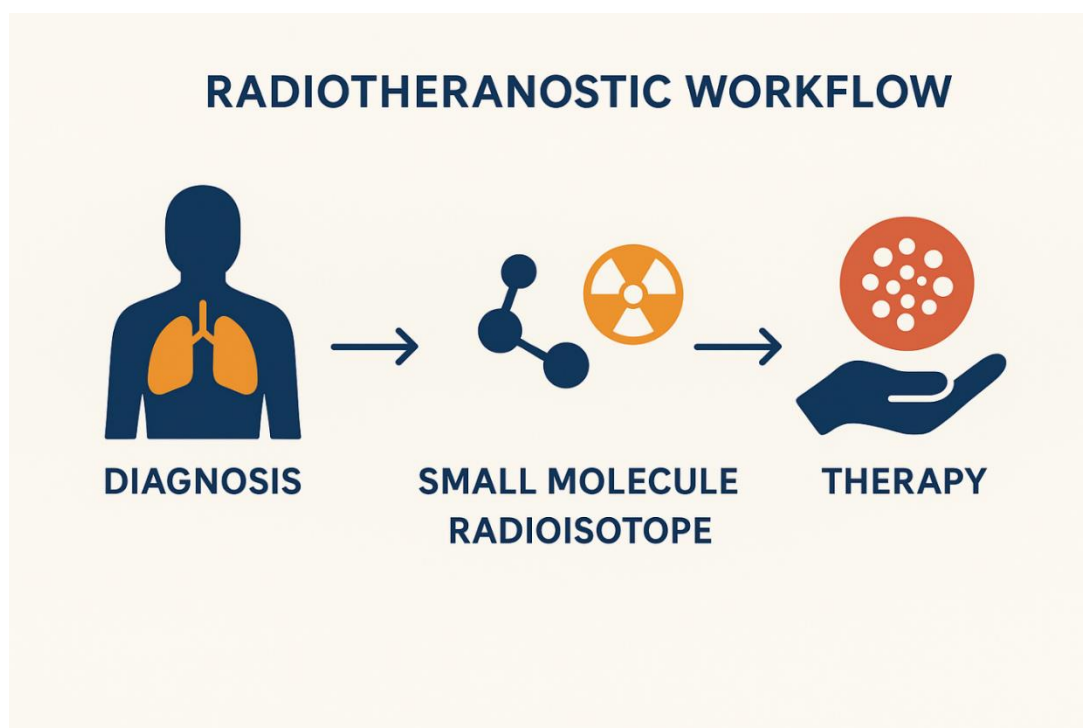


Fig 3: Mechanism of Radiotheranostic Workflow. This diagram illustrates the process by which small molecules, conjugated to radioisotopes, target tumor-associated antigens for both diagnostic

imaging and therapeutic radiation delivery. The integration of these dual functions exemplifies the precision and potential of radiotheranostics in personalized oncology treatments.

Clinical Success Stories

The most convincing proof of radiotheranostics may come from prostate cancer. Prostate-specific membrane antigen (PSMA) is present in large amounts on cancerous prostate cells, making it a great target. In phase III trials, lutetium-177-PSMA-617 showed great effectiveness in males with metastatic castration-resistant prostate cancer, leading to both tumour shrinkage and a significant survival advantage. The integration of diagnostic imaging utilising PSMA PET tracers with the therapeutic administration of ¹⁷⁷Lu has set a novel standard of care in advanced illness (25).

The use of somatostatin receptor-targeted analogues in neuroendocrine tumours is another important example. Lutetium-177-DOTATATE is a radiolabeled peptide that only binds to somatostatin receptor subtype 2, which is seen in large amounts in these tumours. Clinical investigations have demonstrated significant enhancements in progression-

free survival and symptomatic management, accompanied by sustained responses in previously refractory cases.

In addition to these well-known uses, fibroblast activation protein (FAP)-targeted ligands are becoming useful radiotheranostic treatments for solid tumours. FAP is expressed in cancer-associated fibroblasts across many malignancies, establishing it as a universally relevant stromal target. Preliminary investigations utilising FAP-targeted radioligands indicate significant diagnostic sensitivity and therapeutic potential. These clinical achievements demonstrate the versatility of radiotheranostic methods in various oncological settings (26). Table 3 shows that a number of radiotheranostic medicines have been very successful in treating certain types of cancer. This table shows the main drugs, the tumours they target, and their clinical outcomes. It gives real-life instances of how radiotheranostics are changing oncology.

Table 3. Clinical Success Stories in Radiotheranostics:

Agent	Cancer Type	Clinical Outcome
PSMA-617(27)	Prostate Cancer	Tumor regression and improved survival in metastatic castration-resistant prostate cancer
Lutetium-177-DOTATATE(28)	Neuroendocrine Tumors	Significant improvement in progression-free survival and symptomatic control

Advantages

Radiotheranostics have a number of benefits over standard treatments. Using them as companion diagnostics lets doctors choose the right patients: only those who show tracer uptake are treated, which cuts down on unnecessary exposure. Real-time imaging of radiotracer distribution gives us information about pharmacokinetics and tissue biodistribution, which helps with personalised dosing and lessens off-target effects. Moreover, the capacity to visualise tumour uptake before and throughout therapy facilitates adaptive treatment techniques, enabling doctors to customise regimens based on observed tumour biology. This integration of diagnosis, treatment planning, and therapy on a single platform demonstrates the fundamental promise of precision medicine in oncology (29).

Limitations

Radiotheranostics provide certain benefits, but they also have some practical and biological problems. To make and send out therapeutic radioisotopes like Lutetium-177, you need particular infrastructure and a dependable supply chain, which aren't always available around the world. Another issue is off-target

accumulation, which is when tracer uptake happens in places where it shouldn't, such as the salivary glands and kidneys. This might cause xerostomia or renal toxicity. Furthermore, not all tumours exhibit adequate quantities of the targeted antigen to attain treatment success, hence constraining universal application. These issues underscore the necessity for continuous enhancement in ligand design, isotope chemistry, and supportive care measures to broaden safe and equitable access to radiotheranostic therapy (24).

Next-Generation Radiotheranostics

The next generation of radiotheranostics is already starting to take shape. Alpha-emitting isotopes like Actinium-225 and Thorium-227 are more powerful because they emit high-energy, short-range particles that disrupt DNA in a way that can't be fixed while leaving nearby tissues unharmed. Scientists are working on bispecific ligands that can bind to two different targets at the same time. This will make tumours more specific and help them deal with antigen heterogeneity. Finally, improvements in computational chemistry and artificial intelligence are speeding up the process of making ligand scaffolds better, which helps both tumour uptake and pharmacokinetics. These new

ideas point to a future where radiotheranostics becomes a very flexible, patient-specific platform that works well with other precision oncology methods (30).

Integrative Perspectives: Synergizing Modalities

The advent of molecular glues, covalent inhibitors, and radiotheranostics signifies a significant augmentation of the treatment arsenal in oncology. These modalities are not mutually exclusive; instead, they are highly complementary, presenting new options for logical combinations and synergistic methods. For instance, molecular glues that break down oncogenic transcription factors could be used with immune checkpoint inhibitors to change the tumour microenvironment and boost antitumor immunity (31). Covalent inhibitors and targeted degraders can also be used together against kinases to stop adaptive resistance by using different ways to stop it. Radiotheranostics, by inflicting specific DNA damage, could be integrated with inhibitors of DNA damage response pathways (e.g., PARP or ATR inhibitors), enhancing therapeutic efficacy through synthetic lethality (30).

It will be important to use systems biology and network-based methods to map these relationships that function together. Researchers can find weaknesses that

certain types of treatments or combinations of treatments may be best able to exploit by combining multi-omics datasets with computational models. This framework facilitates a more predictive methodology for therapeutic design, advancing from empirical combinations to mechanism-driven solutions.

These new methods work together to make the druggable proteome bigger, which means that it can now target things that were previously "undruggable," like transcription factors, scaffolding proteins, and stromal components. Furthermore, their convergence indicates the potential for modular therapeutic platforms, wherein the selection of chemical modality is customised to the tumor's biology, the vulnerabilities identified through systems-level research, and the clinical setting. This view redefines oncology not as a binary opposition of inhibitors and biologics, but as a spectrum of small-molecule methods, each adaptable to distinct molecular architectures and therapeutic goals. This integrative approach has the potential to reshape the future of precision oncology (32).

Challenges and Future Directions

Safety and Selectivity

Even while they show promise, new small-molecule modalities create serious safety issues. Molecular glues can cause unexpected degradation of substrates, as demonstrated by the teratogenic effects of thalidomide, highlighting the challenges in forecasting degradome scope. Covalent inhibitors, while meticulously engineered, may interact with unexpected nucleophilic residues, resulting in permanent toxicity and possible immunogenicity. Radiotheranostics come with their own set of dangers, such as radiation damage to the salivary glands, kidneys, or bone marrow. To make sure that the advantages of treatment are greater than the risks for different groups of patients, careful engineering of selectivity and long-term pharmacovigilance will be necessary (33).

While these risks are significant, they also highlight avenues for improvement. Engineering greater substrate specificity, designing reversible covalent chemistries, and developing protective co-therapies could mitigate these limitations and enable safer clinical translation.

Manufacturing and Global Access

Another problem is that production is complicated. For example, radiotheranostics need isotopes with short half-lives, which means they need

to be close to nuclear reactors or cyclotrons. These are not evenly spread out over the world. This weak supply chain makes it harder to get things, especially in low- and middle-income countries (LMICs). To keep molecular glues and covalent inhibitors reproducible and cheap, we also need to make progress in chemical synthesis. To fix these unfair situations, the world will need to invest in radiopharmaceutical infrastructure, make regulations easier to follow, and encourage public-private partnerships that put equal access first. This will make sure that advances in chemical oncology are not limited to health systems with a lot of resources (34).

Predictive Biomarkers

As these medicines progress, reliable biomarkers are essential for patient selection and monitoring. For molecular glues, the degrees of ligase expression and the state of mutations will affect how well they break down and how well they work as drugs. For covalent inhibitors, molecular diagnostics like KRAS mutation testing or BTK resistance profiling help doctors decide how to utilise them. Radiotheranostics uniquely benefit from companion imaging modalities such as PSMA PET, which simultaneously predicts therapeutic uptake and efficacy (35). The advancement of biomarkers in the future will necessitate the

amalgamation of multi-omics platforms and longitudinal monitoring, hence enabling a more accurate correlation between patient biology and therapeutic modalities (36).

Future Direction

Integration with cutting-edge technologies may speed up progress in the future. Artificial intelligence and proteomics can help find targets faster, guess which substrates will be degraded, and improve ligand design. Hybrid modalities are coming soon. For example, covalent degraders combine irreversible

binding with induced degradation, while radioglucosides combine metabolic targeting with radionuclide payloads. Ultimately, these discoveries will come together in precision oncology ecosystems that combine small medicines with immunotherapy, gene editing, and cell-based approaches. This forward-thinking model sees a modular and flexible therapeutic environment that will change oncology through chemical creativity and personalised treatment at the systems level (37).

Conclusion

The evolution of small-molecule therapies in oncology signifies a significant conceptual transformation: transitioning from the conventional model of direct enzyme inhibition to more adaptable approaches that regulate, degrade, or accurately distribute therapeutic agents. Molecular glues show how chemical scaffolds can change the way cells work to get rid of proteins that were thought to be "undruggable" before. Covalent inhibitors, on the other hand, stop oncogenic drivers for a long time by being very precise with chemicals. Radiotheranostics, on the other hand, combine diagnostic and treatment into one system, allowing for real-time surveillance of how well treatment is working and where it is going (8,38).

However, the final test for these technologies is still whether they can be used to help people in real life. The real effect of these changes will depend on how

long they last, how well they can get beyond resistance, and how well they can be used in places other than well-funded centres. This necessitates a dual emphasis: scientific innovation to expand the druggable proteome, and health-system foresight to guarantee equitable implementation.

In practical terms, these modalities could expand therapeutic options for resistant and hard-to-treat cancers, offering clinicians tools that go beyond the limits of inhibition. Future research should prioritise biomarker-guided patient selection, global manufacturing solutions, and integration with immuno-oncology. Together, these steps will determine whether the promise of molecular glues, covalent inhibitors, and radiotheranostics translates into durable and equitable improvements in cancer care (39).

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References

1. Baldi S, Long N, Ma S, Liu L, Al-Danakh A, Yang Q, et al. Advancements in Protein Kinase Inhibitors: From Discovery to Clinical Applications. Research (Wash D C). 2024;8:0747. doi: [10.34133/research.0747](https://doi.org/10.34133/research.0747).
2. Singh N, Vayer P, Tanwar S, Poyet JL, Tsaïoun K, Villoutreix BO. Drug discovery and development: introduction to the general public and patient groups. Front Drug Discov [Internet]. 2023 May 24 [cited 2025 Aug 23];3. Available

- from: <https://www.frontiersin.org/journals/drug-discovery/articles/10.3389/fddsv.2023.1201419/full>
3. Zhong G, Chang X, Xie W, Zhou X. Targeted protein degradation: advances in drug discovery and clinical practice. *Sig Transduct Target Ther.* 2024 Nov 6;9(1):308. doi: [10.1038/s41392-024-02011-0](https://doi.org/10.1038/s41392-024-02011-0).
 4. Yadav RK, Ali A, Kumar S, Sharma A, Baghchi B, Singh P, et al. CAR T cell therapy: newer approaches to counter resistance and cost. *Heliyon.* 2020 Apr 16;6(4):e03779. doi: [10.1016/j.heliyon.2020.e03779](https://doi.org/10.1016/j.heliyon.2020.e03779).
 5. Qin S, Jiang J, Lu Y, Nice EC, Huang C, Zhang J, et al. Emerging role of tumor cell plasticity in modifying therapeutic response. *Sig Transduct Target Ther.* 2020 Oct 7;5(1):228. doi: [10.1038/s41392-020-00359-5](https://doi.org/10.1038/s41392-020-00359-5).
 6. Huggins DJ, Sherman W, Tidor B. Rational Approaches to Improving Selectivity in Drug Design. *J Med Chem.* 2012 Feb 23;55(4):1424–44. doi: [10.1021/jm2010332](https://doi.org/10.1021/jm2010332).
 7. Fu YC, Liang SB, Luo M, Wang XP. Intratumoral heterogeneity and drug resistance in cancer. *Cancer Cell International.* 2025 Mar 18;25(1):103. doi: [10.1186/s12935-025-03468-9](https://doi.org/10.1186/s12935-025-03468-9).
 8. Sasso JM, Tenchov R, Wang D, Johnson LS, Wang X, Zhou QA. Molecular Glues: The Adhesive Connecting Targeted Protein Degradation to the Clinic. *Biochemistry.* 2022 Jul 20;62(3):601. doi: [10.1021/acs.biochem.2c00246](https://doi.org/10.1021/acs.biochem.2c00246).
 9. Cheke RS, Kharkar PS. Covalent inhibitors: An ambitious approach for the discovery of newer oncotherapeutics. *Drug Development Research.* 2024;85(1):e22132. doi: [10.1002/ddr.22132](https://doi.org/10.1002/ddr.22132).
 10. Herrmann K, Schwaiger M, Lewis JS, Solomon SB, McNeil BJ, Baumann M, et al. Radiotheranostics: a roadmap for future development. *Lancet Oncol.* 2020 Mar;21(3):e146–56. doi: [10.1016/S1470-2045\(19\)30821-6](https://doi.org/10.1016/S1470-2045(19)30821-6).
 11. Asatsuma-Okumura T, Ito T, Handa H. Molecular Mechanisms of the Teratogenic Effects of Thalidomide. *Pharmaceuticals.* 2020 May;13(5):95. doi: [10.3390/ph13050095](https://doi.org/10.3390/ph13050095).
 12. Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, et al. The Myeloma Drug Lenalidomide Promotes the Cereblon-Dependent Destruction of Ikaros Proteins. *Science.* 2014 Jan 17;343(6168):305–9. doi: [10.1126/science.1244917](https://doi.org/10.1126/science.1244917).
 13. Yang Y, Li Z, Yang Y, Xiao P, He Z, Zhang Z, et al. The RBM39 degrader indisulam inhibits acute megakaryoblastic leukemia by altering the alternative splicing of ZMYND8. *Cell Biosci.* 2025 Apr 13;15(1):46. doi: [10.1186/s13578-025-01359-4](https://doi.org/10.1186/s13578-025-01359-4).
 14. Shen Y, Wen W, Chen L, Zhang N, Cong W, Hu H. Recent Advances in Targeted Protein Degradation: Molecular Glues as Anticancer Drugs. *Clin Exp Pharmacol Physiol.* 2025 Sep;52(9):e70064. doi: [10.1111/1440-1681.70064](https://doi.org/10.1111/1440-1681.70064).
 15. Baek K, Metivier RJ, Roy Burman SS, Bushman JW, Yoon H, Lumpkin RJ, et al. Unveiling the hidden interactome of CRBN molecular glues. *Nat Commun.* 2025 Jul 24;16(1):6831. doi: [10.1038/s41467-025-51200-4](https://doi.org/10.1038/s41467-025-51200-4).
 16. Islam NN, Caulfield TR. Conditioned Generative Modeling of Molecular Glues: A Realistic AI Approach for Synthesizable Drug-like Molecules. *Biomolecules.* 2025 Jun;15(6):849. doi: [10.3390/biom15060849](https://doi.org/10.3390/biom15060849).
 17. Kim H, Hwang YS, Kim M, Park SB. Recent advances in the development of covalent inhibitors. *RSC Med Chem.* 2021;12(7):1037–45. doi: [10.1039/d1md00068c](https://doi.org/10.1039/d1md00068c).
 18. Gai C, Harnor SJ, Zhang S, Cano C, Zhuang C, Zhao Q. Advanced approaches to developing targeted covalent drugs. *RSC Med Chem.* 2022;13(12):1460–75. doi: [10.1039/d2md00208c](https://doi.org/10.1039/d2md00208c).
 19. Cox AD, Der CJ. “Undruggable KRAS”: druggable after all. *Genes Dev.* 2025 Jan 1;39(1–2):132–62. doi: [10.1101/gad.351764.125](https://doi.org/10.1101/gad.351764.125).
 20. Rozkiewicz D, Hermanowicz JM, Kwiatkowska I, Krupa A, Pawlak D. Bruton's Tyrosine Kinase Inhibitors (BTKIs): Review of Preclinical Studies and Evaluation of Clinical Trials. *Molecules.* 2023 Mar 6;28(5):2400. doi: [10.3390/molecules28052400](https://doi.org/10.3390/molecules28052400).
 21. Grabe T, Jeyakumar K, Niggenaber J, Schulz T, Koska S, Kleinbölting S, et al. Addressing the Osimertinib Resistance Mutation EGFR-L858R/C797S with Reversible Aminopyrimidines. *ACS Med Chem Lett.* 2023 Apr 18;14(5):591–8. doi: [10.1021/acsmedchemlett.3c00017](https://doi.org/10.1021/acsmedchemlett.3c00017).

22. McAulay K, Bilsland A, Bon M. Reactivity of Covalent Fragments and Their Role in Fragment-Based Drug Discovery. Pharmaceuticals (Basel). 2022 Nov 8;15(11):1366. doi: [10.3390/ph15111366](https://doi.org/10.3390/ph15111366).
23. Kalgutkar AS, Dalvie DK. Drug discovery for a new generation of covalent drugs. Expert Opinion on Drug Discovery [Internet]. 2012 Jul 1 [cited 2025 Aug 23];7(7):561-81. Available from: <https://www.tandfonline.com/doi/abs/10.1517/17460441.2012.688744> doi: [10.1517/17460441.2012.688744](https://doi.org/10.1517/17460441.2012.688744).
24. Bodei L, Herrmann K, Schöder H, Scott AM, Lewis JS. Radiotheranostics in oncology: current challenges and emerging opportunities. Nat Rev Clin Oncol. 2022 Aug;19(8):534–50. doi: [10.1038/s41571-022-00652-y](https://doi.org/10.1038/s41571-022-00652-y).
25. Uemura M, Watabe T, Hoshi S, Tanji R, Yaginuma K, Kojima Y. The current status of prostate cancer treatment and PSMA theranostics. Ther Adv Med Oncol. 2023 Jul 3;15:17588359231182293. doi: [10.1177/17588359231182293](https://doi.org/10.1177/17588359231182293).
26. Advances With 225Ac-DOTATATE Targeted Alpha Therapy in Somatostatin Receptor Positive Neuroendocrine Tumors. Seminars in Nuclear Medicine [Internet]. 2025 Jul 4 [cited 2025 Aug 23]; Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0001299825000728> doi: [10.1053/j.semnuclmed.2025.04.006](https://doi.org/10.1053/j.semnuclmed.2025.04.006).
27. Kalender E, Ekinci E, Elboğa U, Şahin E. Efficacy of 177Lu-PSMA-617 Therapy in mCRPC Patients with Liver Metastases: Insights into Survival Outcomes and Predictors of Response. Biomedicines. 2025 Feb 24;13(3):569. doi: [10.3390/biomedicines13030569](https://doi.org/10.3390/biomedicines13030569).
28. Santos S, Martins FA, P SA, Ferreira G, Lucena I. Treatment of lung neuroendocrine tumors with ¹⁷⁷Lu-DOTATATE: experience from a tertiary oncology centre. Endocrine Abstracts [Internet]. 2025 May 9 [cited 2025 Aug 23];110. Available from: <https://www.endocrine-abstracts.org/ea/0110/ea0110p475>
29. Bednarz B. Theranostics and Patient-Specific Dosimetry. Semin Radiat Oncol. 2023 Jul;33(3):317–26. doi: [10.1016/j.semradonc.2023.03.007](https://doi.org/10.1016/j.semradonc.2023.03.007).
30. Zhang S, Wang X, Gao X, Chen X, Li L, Li G, et al. Radiopharmaceuticals and their applications in medicine. Sig Transduct Target Ther. 2025 Jan 3;10(1):1. doi: [10.1038/s41392-024-01923-z](https://doi.org/10.1038/s41392-024-01923-z).
31. Dewey JA, Delalande C, Azizi SA, Lu V, Antonopoulos D, Babnigg G. Molecular Glue Discovery: Current and Future Approaches. J Med Chem. 2023 Jul 27;66(14):9278–96. doi: [10.1021/acs.jmedchem.3c00449](https://doi.org/10.1021/acs.jmedchem.3c00449).
32. Jiang W, Ye W, Tan X, Bao YJ. Network-based multi-omics integrative analysis methods in drug discovery: a systematic review. BioData Min. 2025 Mar 28;18:27. doi: [10.1186/s13040-025-00403-6](https://doi.org/10.1186/s13040-025-00403-6).
33. Asatsuma-Okumura T, Ito T, Handa H. Molecular Mechanisms of the Teratogenic Effects of Thalidomide. Pharmaceuticals (Basel). 2020 May 13;13(5):95. doi: [10.3390/ph13050095](https://doi.org/10.3390/ph13050095).
34. Wang Y, Chen D, Augusto R dos S, Liang J, Qin Z, Liu J, et al. Production Review of Accelerator-Based Medical Isotopes. Molecules. 2022 Aug 19;27(16):5294. doi: [10.3390/molecules27165294](https://doi.org/10.3390/molecules27165294).
35. Okafor CE, Egwuatu EC, Owosagba VA, Njei T, Adeyemi BI, Onuche PUO, et al. From Bench to Bedside: Medicinal Chemistry Strategies in the Development of Kinase Inhibitors for Cancer Therapy. Journal of Cancer and Tumor International. 2025 May 10;15(2):79–96. doi: [10.9734/jcti/2025/v15i2348](https://doi.org/10.9734/jcti/2025/v15i2348).
36. Bodaghi A, Fattahi N, Ramazani A. Biomarkers: Promising and valuable tools towards diagnosis, prognosis, and treatment of Covid-19 and other diseases. Heliyon. 2023 Jan 30;9(2):e13323. doi: [10.1016/j.heliyon.2023.e13323](https://doi.org/10.1016/j.heliyon.2023.e13323).
37. Duran-Frigola M, Cigler M, Winter GE. Advancing Targeted Protein Degradation via Multiomics Profiling and Artificial Intelligence. J Am Chem Soc. 2023 Jan 27;145(5):2711–32. doi: [10.1021/jacs.2c11098](https://doi.org/10.1021/jacs.2c11098).
38. Onwuemelem LA, Orobator ET, Onyedum NN, Chibueze ES, Kanu I, Christopher AA, et al. Molecular Mechanisms of Clonal Hematopoiesis in Age-Related Cardiovascular Disease and Hematologic Malignancies:

Review Article. Journal of Pharma Insights and Research. 2025 Apr 5;3(2):358–72. doi: [10.69613/jpir32.2025.358](https://doi.org/10.69613/jpir32.2025.358).

39. Lawal OP, Orobator ET, Dorcas SI, Ezeamii VC, Foster-Pagaebi E, Attahiru M. Orphan Drug Development for Rare Diseases: Therapeutic Challenges, Translational Strategies, and Global Health Equity. Journal of Life Science and Public Health. 2025 Jun 30;1(1):1–9. doi: [10.69613/jlsph11.2025.1](https://doi.org/10.69613/jlsph11.2025.1).

Review Article

Biofilms in Resource-Limited Settings: Challenges, Opportunities, and Innovative Solutions in Nigeria

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Abstract:

Microbial biofilms represent a growing yet often overlooked public health concern, particularly in resource-limited settings where they exacerbate the burden of persistent infections and antimicrobial resistance (AMR). In Nigeria, fragile healthcare infrastructure, poor funding, and a continuous loss of skilled medical professionals compound the difficulty of managing biofilm-associated infections. These microbial communities, embedded in self-produced extracellular matrices, exhibit remarkable resistance to antimicrobials and host immune defenses, leading to chronic and recurrent infections that further strain an already overstretched health system. This review synthesizes evidence published between 2010 and 2025 to examine the burden, challenges, and opportunities surrounding biofilm control in Nigeria. Literature was systematically retrieved from PubMed, Scopus, and Google Scholar, complemented by reports from WHO and the Nigerian Centre for Disease Control. Findings indicate that biofilms not only complicate clinical treatment outcomes but also persist in environmental reservoirs, particularly water systems, serving as hidden amplifiers of resistance and infection transmission. To address these challenges, the review explores low-cost and context-appropriate strategies such as harnessing Nigeria's biodiversity for the discovery of plant-derived antibiofilm compounds, implementing decentralized engineering solutions for water treatment, and promoting community-based infection prevention initiatives. It further emphasizes the importance of local innovation, interdisciplinary collaboration, and policy support within a One Health framework that integrates human, animal, and environmental health. By spotlighting the Nigerian experience, this review calls for urgent investment and global attention to biofilm-related infections in Low- and Middle-Income Countries (LMICs).

Keywords: Biofilms; Antimicrobial Resistance; Nigeria; One Health; LMICs; Water Systems; Public Health

Introduction

Biofilms represent a pervasive and complex challenge in global health, impacting various sectors from clinical medicine to environmental management. These intricate microbial communities, characterized by their adherence to surfaces and encapsulation within a self-produced extracellular polymeric substance (EPS) matrix, confer remarkable protection and adaptability to microorganisms (1). This structural organization enhances microbial survival, allowing them to thrive in diverse environments (1, 2). While biofilms can exist in neutral or even beneficial contexts, their role in disease is profound, accounting for an estimated 70% of all microorganism-induced infections globally, with specific national estimates for Nigeria remaining scarce (3).

These infections, often nosocomial and chronic, contribute significantly to patient morbidity and mortality. The protective EPS matrix and altered physiological states within biofilms render the embedded microorganisms highly resistant to conventional antimicrobial agents, often exhibiting resistance levels up to 1000-fold higher than their planktonic counterparts, and effectively evading host immune responses (3, 4).

The challenges posed by biofilms are particularly acute in Low- and Middle-Income Countries (LMICs), where existing healthcare system vulnerabilities amplify their detrimental effects (3). Nigeria, with its large population and significant health disparities, serves as a critical example of this amplified burden. The nation grapples with a high prevalence of infectious diseases, including malaria, lower respiratory infections, diarrheal diseases, and tuberculosis, which remain among the leading causes of death, with communicable conditions accounting for a substantial majority of fatalities (5).

The Nigerian healthcare system is severely constrained by multiple systemic challenges. Infrastructure remains inadequate, marked by outdated facilities, overcrowding, limited modern equipment, frequent drug shortages, unreliable power supply, and insufficient access to clean water (6, 7). Financial investment is notably low, with less than 5% of the national budget allocated to healthcare, far below the 15% target recommended by the African Union's Abuja Declaration (7). Consequently, most Nigerians rely on out-of-pocket payments, leaving fewer than 10%

covered by health insurance (6, 7). A critical shortage of medical professionals, worsened by a persistent "brain drain" as skilled personnel migrate abroad for better opportunities, further weakens service delivery (6, 8). The doctor-to-patient ratio in some states is as high as 1:3,500, compared with the World Health Organization's recommended 1:600 (7). These resource gaps undermine infection prevention and control (IPC) practices, with inadequate personal protective equipment (PPE) and IPC materials frequently cited as barriers to compliance (8, 9); overcrowding and a lack of isolation rooms in healthcare facilities further facilitate the spread of healthcare-associated infections (10).

The inherent complexity and resistance of biofilms make them formidable adversaries even in well-resourced healthcare systems. When combined with the systemic deficiencies prevalent in LMICs like Nigeria, the burden of biofilm-associated infections often becomes an unacknowledged or "hidden epidemic" (6). The underlying biofilm nature of persistent infections frequently goes undiagnosed or mismanaged, resulting in prolonged illness, increased morbidity and mortality, and additional strain on already overwhelmed healthcare systems. The true scale of the problem is likely underestimated due to these compounding factors.

Given the convergence of a high infectious disease burden, weak healthcare infrastructure, and the documented presence of antibiotic-resistant biofilms in both clinical and environmental settings, Nigeria represents a critical yet understudied case. While other LMICs, such as India, Kenya, and Bangladesh, have begun advancing targeted biofilm research and interventions (11), Nigeria still lacks a unified synthesis linking biofilm biology, systemic fragility, and antimicrobial resistance. This review addresses that gap by integrating biological, environmental, and policy perspectives within a One Health framework. It identifies context-appropriate, low-cost strategies to mitigate biofilm-associated infections and emphasizes the need for local innovation and interdisciplinary collaboration. By spotlighting Nigeria, the review contributes insights relevant to other resource-limited settings. Figure 1 presents the conceptual framework, while Table 1 outlines specific healthcare system challenges influencing biofilm control in Nigeria.

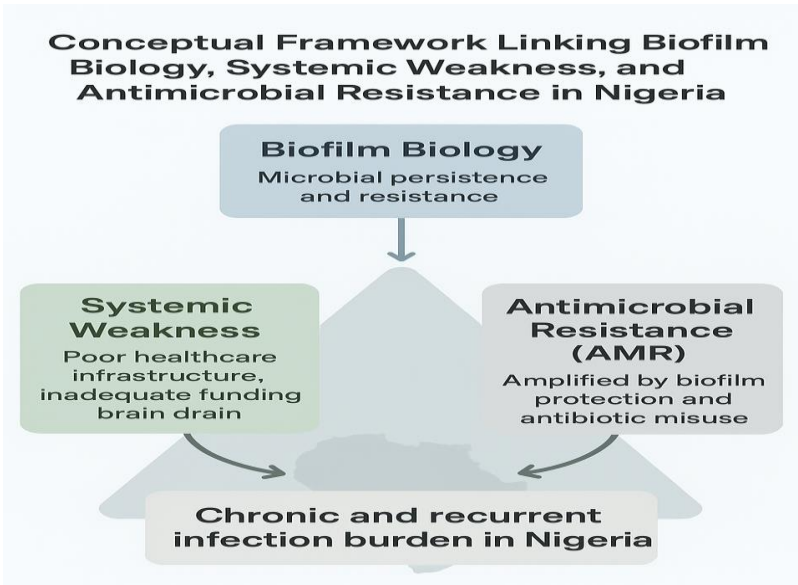


Figure 1. Conceptual framework linking biofilm biology, systemic weaknesses, and antimicrobial resistance leading to chronic infection burden in Nigeria.

Table 1: Healthcare System Challenges in Nigeria and Their Impact on Biofilm Infection Control

Healthcare System Challenge	Specific Impact on Biofilm Infection Control	Relevant Statistics/Examples
Inadequate Healthcare Infrastructure	Limited access to diagnostic equipment for biofilm detection; compromised sterilization and maintenance of medical devices; increased HAIs due to poor facility conditions.	Lack of modern equipment, overcrowding, outdated facilities, poor maintenance, power outages, and lack of clean water (7).
Poor Healthcare Funding	Inability to afford advanced biofilm treatments (e.g., novel antibiofilm agents, specialized equipment); low investment in medical research for local solutions.	Less than 5% of the national budget is allocated to healthcare (7). Public financing covers only 25% of total health spending (12).
Shortage of Medical Professionals & Brain Drain	Reduced capacity for specialized care and research in biofilm-related diseases; increased workload on remaining staff, potentially impacting IPC compliance.	Doctor-to-patient ratio 1:3,500 vs. WHO 1:600 (8). Over 50% of Nigerian doctors work abroad (7).
High Disease Burden	Overwhelmed healthcare facilities; diversion of resources to acute infectious diseases, potentially neglecting chronic biofilm infections.	Malaria, lower respiratory infections, diarrhea diseases, and tuberculosis are leading causes of death (5).
Poor Health Insurance Coverage	Financial burden on patients leading to delayed or incomplete treatment for persistent biofilm infections; reliance on self-medication or unqualified alternatives.	Less than 10% of Nigerians are covered by NHIS (7, 12).
Inadequate Infection Prevention & Control (IPC) Practices	Increased spread of biofilm-associated HAIs; difficulty in implementing effective control measures.	70.5% of doctors and 67.0% of students identified inadequate PPE supply as a barrier (8, 9). IPC materials 78.9% "always not available"(10).
Overcrowding/Lack of Isolation Rooms	Facilitates rapid transmission of biofilm-forming pathogens within healthcare settings.	Overcrowding and lack of isolation rooms are known risk factors for HAIs (10).

Methodology

Literature Search Strategy

A comprehensive literature search was conducted between January and June 2025 to gather pertinent peer-reviewed articles, reports, and grey literature on microbial biofilms in resource-limited settings (RLS), with a specific focus on Nigeria. This review focused on biofilms relevant to human health and associated environmental reservoirs that influence infection transmission in Nigeria. The search was primarily conducted across major scientific and public health databases, including PubMed, Scopus, and Google Scholar, and was supplemented by examining grey literature from authoritative sources such as the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), the Nigerian Centre for Disease Control and Prevention (NCDC), and relevant environmental health agencies.

The core search strategy utilized combinations of keywords and Boolean operators to ensure comprehensive coverage across the paper’s three thematic areas: challenges, opportunities, and solutions. Key terms included: (Biofilm OR Biofilms OR "Biofilm-associated infection") AND (Nigeria OR "Sub-Saharan Africa" OR "Low- and Middle-Income Countries" OR LMICs OR "Resource-Limited Settings") AND ("Antimicrobial Resistance" OR AMR OR "Chronic Infection" OR Persistence OR Control OR Management) AND ("Water Sanitation" OR "Decentralized Solution" OR "Plant-Derived Agents" OR "Natural Products" OR "Community Health") AND ("One Health"). These terms were strategically combined (e.g., (Biofilm AND Nigeria AND AMR); (LMICs AND "Plant-Derived Agents" AND Biofilm); ("One Health" AND Biofilm AND Water)) to retrieve relevant records.

All records retrieved were exported to a reference management software (EndNote 2024) for organization and duplicate removal, and all searches were restricted to articles written in the English language. Two independent researchers initially screened the titles and abstracts of the remaining records for relevance to the review's scope. Full-text articles were then retrieved and assessed for eligibility by the same researchers, with any disagreements resolved through consensus or arbitration from a third reviewer.

Inclusion and Exclusion Criteria

Inclusion Criteria

The selection process was guided by strict inclusion criteria: (1) Language and Publication Date: Articles published in the English language from 2010 to 2025. (2) Subject Focus: Studies, reviews, or reports focusing explicitly on microbial biofilms in human clinical, animal, or environmental contexts. (3) Thematic Relevance: Content that directly addressed the challenges, systemic barriers in Resource-Limited Settings (RLS), or proposed innovative, low-cost solutions adaptable to Nigeria or similar contexts.

Exclusion Criteria

The following criteria led to the exclusion of records: (1) Topical Irrelevance: Studies entirely unrelated to microbial biofilms, antimicrobial resistance, or public health in RLS. (2) Quality and Source: Articles lacking verifiable peer-review (unless originating from designated authoritative public health bodies like WHO or NCDC). (3) Translational Gap: Laboratory studies that did not discuss the practical implications or translational potential for RLS. The authors thoroughly examined the title, abstract, material and methodology, results, and discussion of the selected articles to extract relevant information.

Table 2. Summary of Included Studies on Biofilms in Nigeria and Related Resource-Limited Settings

Author (Year)	Setting	Biofilm Source/Organism	Key AMR Findings	Relevance/Remarks
Olalemi et al. (2019)	Groundwater, Ado-Ekiti	<i>E. coli</i> , <i>S. faecalis</i>	High resistance to pefloxacin, septrin, chloramphenicol, augmentin	Waterborne AMR source
Agbabiaka et al. (2021)	Treated water, Ilorin	<i>S. aureus</i> , <i>P. aeruginosa</i>	Complete resistance to ceftriaxone, tetracycline	Environmental health risk
Yaki et al. (2024)	Hospital, Nigeria	Uropathogenic <i>E. coli</i>	Strong biofilm formation, multidrug resistance	Clinical AMR link

Umar et al. (2024)	Burn & wound isolates	<i>S. aureus</i> (MRSA)	mecA gene detected, biofilm producer	Healthcare-associated risk
Nwankwo et al. (2025)	Oral biofilm (children)	<i>A. caviae</i>	Sensitive to plant extract-nanoparticle combo	Innovation potential

Search Outcome and Screening Process

A total of 205 records were retrieved from databases and grey literature sources. After duplicate

removal and screening, 103 full-text articles were reviewed in detail, and 41 studies met the inclusion criteria for narrative synthesis, of which 32 are cited.

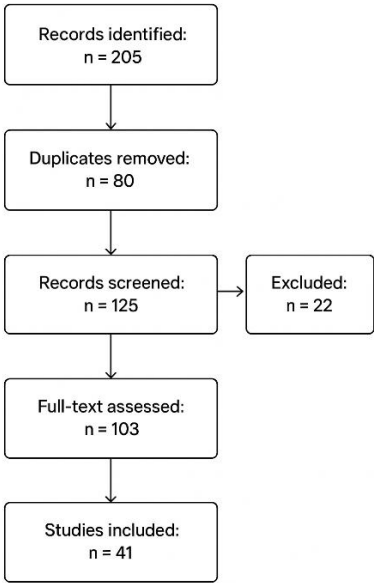


Figure 2. PRISMA-style summary of the literature selection and inclusion process.

Data Extraction and Quality Appraisal

Data were extracted from all included studies using a standardized template summarizing authorship, publication year, study setting, type of biofilm or organism investigated, antimicrobial resistance findings, and key contextual relevance to Nigeria. The quality of peer-reviewed empirical studies was appraised using the Joanna Briggs Institute (JBI) critical appraisal checklist, while grey literature (e.g., WHO and NCDC reports) was assessed using the AACODS checklist for authority, accuracy, coverage, objectivity, date, and significance. Discrepancies in assessment were resolved through consensus between two reviewers. Due to the heterogeneity of study types and outcomes, no quantitative synthesis or meta-analysis was undertaken.

Data Synthesis

Given the inherent heterogeneity of the included literature, which spans clinical microbiology, public health policy, and environmental engineering, a

narrative synthesis approach was adopted. This method involved systematically integrating and synthesizing both qualitative and quantitative findings to build a cohesive and comprehensive argument across the review. The synthesis was intentionally structured around three distinct thematic areas that reflect the abstract's scope: (1) Biofilm Pathogenesis and Antimicrobial Resistance in Nigerian Healthcare, which addresses the clinical and systemic challenges; (2) Environmental Biofilms and Public Health in Nigeria: A "One Health" Perspective, which links clinical burdens to environmental reservoirs like water; and (3) Low-Cost Strategies and Innovative Opportunities for Biofilm Control in LMICs, which focuses on solutions, including leveraging local biodiversity and decentralized engineering. No quantitative meta-analysis was performed due to the diverse nature of the study designs and outcomes.

Biofilm Pathogenesis and Antimicrobial Resistance in Nigerian Healthcare

Here, we explore the intricate mechanisms by which biofilms contribute to persistent infections and antimicrobial resistance within the Nigerian healthcare landscape, underscoring the diagnostic and treatment challenges encountered.

Mechanisms of Biofilm Formation and Their Role in Persistent Infections

Biofilm formation (Figure 3) is a sophisticated, multi-stage biological process that enables microbial communities to establish and persist (1, 13). It typically commences with a reversible attachment phase, where bacteria non-specifically adhere to a surface. This is followed by an irreversible attachment phase, mediated by bacterial adhesins and structures like fimbriae (1, 2). Subsequently, the maturation phase involves the extensive production of an extracellular polymeric substance (EPS), which forms a protective matrix around the cells. Within this matrix, the biofilm develops a complex social structure, often including permeable water channels that facilitate nutrient and waste transport. Finally, individual cells or clusters can detach from the mature biofilm, dispersing to colonize new sites (4).

The EPS matrix is a critical component, acting as a formidable physical barrier that impedes the penetration of antibiotics and host defense molecules, while also providing protection against various environmental stressors such as UV radiation, pH fluctuations, and desiccation (3). Biofilm formation initiates disease processes through several mechanisms, including the detachment of individual bacterial cells or

aggregates, the production of endotoxins, enhanced evasion of host immune surveillance, and the establishment of a protective environment conducive to the emergence of more virulent phenotypes (4, 13). This inherent protective capacity makes biofilm-associated infections notoriously chronic and difficult to treat, leading to increased morbidity, higher mortality rates, and substantial healthcare costs (1, 4). Clinically, biofilm-associated infections can be broadly categorized into device-associated biofilms (e.g., catheters, ventilators, prosthetic implants) and chronic tissue biofilms (e.g., wounds, chronic otitis, cystic fibrosis infections). This distinction is clinically important because prevention and treatment strategies differ - while the former may require device modification or removal, the latter often necessitate prolonged combination therapy or biofilm-targeted agents (3).

Biofilms are a powerful survival strategy for microorganisms, allowing them to persist against host defenses and antimicrobial treatments. In Nigeria, where healthcare resources are severely limited, the ability of pathogens to form biofilms creates a compounding problem. Persistent infections lead to longer hospital stays and recurrent treatments, draining already scarce resources (11). This means that the biological advantage conferred by biofilm formation directly translates into a significant economic and logistical burden, trapping the healthcare system in a cycle of managing chronic, difficult-to-eradicate infections rather than effectively preventing them.

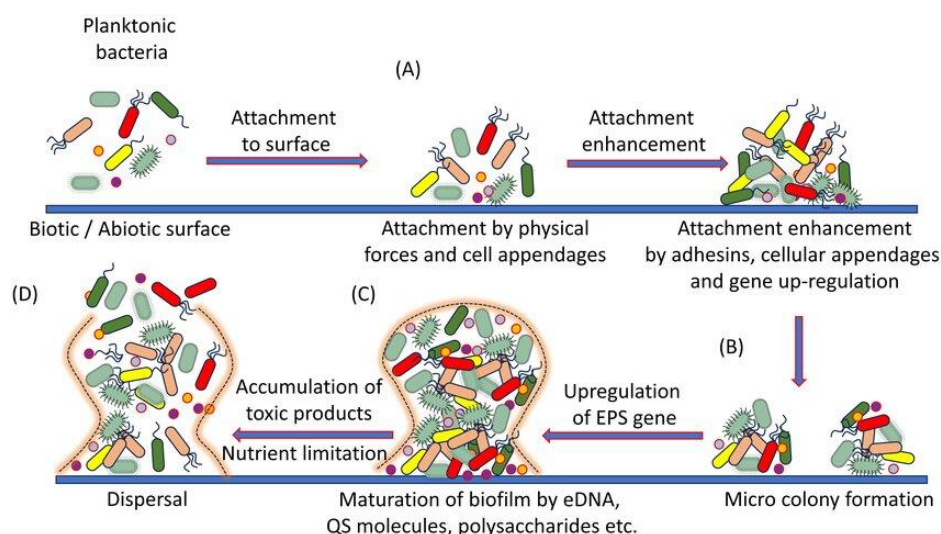


Figure 3: Mechanism of Biofilm Formation. The four stages include: (A) Initial Attachment: Free-living bacteria reversibly attach to a surface, progressing to irreversible binding via adhesins. (B) Microcolony Formation: Bacteria multiply and cluster, upregulating genes for maintained adherence. (C) Biofilm Maturation: Cells produce and are encased by the thick, protective Extracellular Polymeric Substances (EPS) matrix. Quorum Sensing (QS) regulates the structure. (D) Dispersal: Nutrient depletion and waste buildup trigger the release of bacteria, allowing colonization of new sites. Adapted from:(2).

The Escalating Crisis of Antimicrobial Resistance (AMR) in Nigeria, Exacerbated by Biofilm Presence

Antimicrobial resistance (AMR) constitutes a critical public health challenge in Nigeria, a crisis significantly amplified by the widespread presence of biofilms. The problem is exacerbated by extensive antibiotic use across various sectors, coupled with improper empirical prescriptions and ineffective antimicrobial stewardship programs (14). High antibiotic resistance patterns are consistently detected in bacterial isolates recovered from healthcare settings, food supply chains, and environmental sources across the country (14, 15).

Studies in Nigeria have provided concrete evidence of this link. For instance, bacterial isolates from biofilms in groundwater sources in Ado-Ekiti, including *Streptococcus faecalis*, *Escherichia coli*, and *Staphylococcus aureus*, exhibited high resistance to common antibiotics such as pefloxacin, septrin, chloramphenicol, and augmentin (16). Similarly, biofilm-producing bacteria isolated from treated water supplies in Ilorin displayed complete resistance to ceftriaxone, amoxicillin, tetracycline, and cotrimoxazole, further suggesting that biofilm counts could serve as indicators of water quality (17). In clinical settings, strong biofilm-forming uropathogenic *E. coli* isolates from catheterized patients in a Nigerian hospital were found to be extensively drug-resistant, demonstrating a significant correlation between biofilm formation and resistance to multiple antibiotics, including Augmentin, Ceftazidime, Gentamicin, and Ciprofloxacin (18).

Furthermore, *Staphylococcus aureus* isolates from burn and wound patients and healthcare workers

in Northern Nigeria were identified as moderate biofilm producers, with half of these isolates carrying the *mecA* gene, indicative of Methicillin-Resistant *Staphylococcus aureus* (MRSA) (19). The persistence of "ESKAPE" organisms (e.g., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), which are frequently associated with multiple antibiotic resistance and biofilm formation, creates an especially challenging scenario in intensive care units (ICUs), where multiple resistant pathogens coexist (19, 20). These findings highlight that biofilm-producing pathogens are not only widespread across hospital environments but also deeply embedded in Nigeria’s AMR crisis, where diagnostic infrastructure rarely accounts for biofilm presence.

Nigeria already faces a dire AMR crisis due to systemic issues like poor regulation and misuse of antibiotics. The presence of biofilms, which inherently increase bacterial resistance through physical protection and altered growth rates, acts as an accelerator of this existing AMR problem. This means that even if a bacterial strain is initially susceptible to an antibiotic, its ability to form a biofilm in the body or environment can render standard treatments ineffective, thereby accelerating the selection and spread of multidrug-resistant strains. This dynamic makes the AMR challenge in Nigeria exponentially more difficult to combat, demanding strategies that specifically target the biofilm state rather than solely focusing on planktonic bacteria. Table 3 summarizes the most frequently reported biofilm-forming pathogens in Nigeria, their typical sources, and associated resistance patterns.

Table 3: Key Biofilm-Associated Pathogens and Their Resistance Patterns in Nigerian Clinical and Environmental Settings

Pathogen	Source/Context	Biofilm-Forming Capacity	Key Antibiotic Resisted	Relevant Snippets
<i>Staphylococcus aureus</i> (MRSA, MSSA, CoNS)	Wastewater, Surface water, Burn/Wound patients, Healthcare workers	High (environmental); Moderate (clinical)	Varies by strain; MRSA is a major concern	(21)
<i>Escherichia coli</i> (Uropathogenic <i>E. coli</i>)	Groundwater, Treated Water Distribution Systems (DWDS), Catheterized patients, Raw meat	High (environmental); Strong (clinical)	Pefloxacin, Septrin, Chloramphenicol, Augmentin, Ceftazidime, Ceftriaxone, Gentamicin, Ciprofloxacin, Ampicillin, Gentamicin	(18)
<i>Pseudomonas aeruginosa</i>	Oral cavity, Hospital environment, Treated	Biofilm producer	Gentamicin, Amoxicillin-clavulanic acid, Cefixime	(17)

	Water Distribution Systems (DWDS)			
<i>Streptococcus faecalis</i>	Groundwater	Biofilm producer	Multiple antibiotic resistance (MAR)	(16)
<i>Enterobacter aerogenes</i>	Groundwater, Hospital environment	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
<i>Proteus mirabilis</i>	Groundwater, Oral cavity	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
<i>Salmonella typhi</i>	Groundwater	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
<i>Shigella dysenteriae</i>	Groundwater, Treated Water Distribution Systems (DWDS)	Biofilm producer	Pefloxacin, Septrin, Chloramphenicol, Augmentin	(16)
<i>Aeromonas caviae</i>	Oral cavity	Biofilm producer	Not specified, but anti-infective nanoparticles/plant extracts are effective	(22)
<i>Serratia marcescens</i>	Oral cavity, Hospital environment, Treated Water Distribution Systems (DWDS)	Biofilm producer	Not specified, but anti-infective nanoparticles/plant extracts are effective	(17)

Challenges in Diagnosis and Treatment of Biofilm-Associated Infections in Resource-Constrained Healthcare Settings

The effective management of biofilm-associated infections in Nigeria is severely hampered by significant diagnostic and treatment challenges, exacerbated by resource limitations. A primary obstacle is the difficulty in culturing biofilms in laboratory settings, coupled with their inherent complexity and high tolerance to conventional methods (23, 24). This often leads to a critical diagnostic gap: routine tests frequently do not include specific biofilm detection or comprehensive antimicrobial susceptibility assessments, which are crucial for guiding appropriate antibiotic treatment (18). This omission reflects the broader reality that biofilm detection is not part of routine microbiological diagnostics in most Nigerian laboratories, primarily due to limited equipment, lack of training, and absence of standardized protocols (18, 24). Consequently, clinicians may treat infections based on the susceptibility of planktonic (free-floating)

bacteria, unaware that the underlying biofilm structure renders these treatments ineffective (25). This leads to repeated treatment failures, prolonged patient suffering, increased healthcare costs, and ultimately contributes to the proliferation of untreatable infections. Addressing this diagnostic blind spot with accessible, low-cost methods is therefore vital for improving patient outcomes and combating AMR (11).

The high frequency of device-associated infections, such as catheter-associated urinary tract infections (CAUTIs), and the persistence of biofilm-driven infections in intensive care units (ICUs) highlight the pervasive nature of the problem (18, 23). While innovative technologies are warranted to manage the complexities presented by these biofilm infections, such advancements are often unavailable or unaffordable in LMICs (20). Despite ongoing efforts by healthcare providers and policymakers, overcoming biofilm formation in settings like ICUs remains a persistent challenge, contributing significantly to the burden of chronic infections (20).

Environmental Biofilms and Public Health in Nigeria: A "One Health" Perspective

In this section, we broaden the scope to examine environmental biofilms, emphasizing their profound role in public health within Nigeria through the lens of the "One Health" approach, and considering the exacerbating factors such as climate change.

Prevalence and Impact of Environmental Biofilms on Public Health

Biofilms are ubiquitous in various environmental sectors, including wastewater and surface waters, where their presence can significantly compromise public health (21). Environmental factors such as temperature, pH, nutrient content, salinity, and dissolved oxygen are critical determinants influencing biofilm formation, growth, and persistence (21, 25). These risks are expected to intensify under changing

climatic conditions, where increased temperature, flooding, and drought create favorable conditions for biofilm persistence and antibiotic resistance dissemination (21, 26).

Studies conducted in Nigeria have revealed the widespread presence of antibiotic-resistant bacteria within biofilms in groundwater sources, including boreholes and wells. These findings indicate substantial contamination risks and the potential for widespread waterborne diseases (16). Drinking water distribution systems (DWDS) in Nigeria are particularly concerning, as they can serve as active incubators for the proliferation and dissemination of antibiotic-resistant opportunistic pathogens. Biofilm growth within these systems can visibly alter water quality, affecting turbidity, taste, color, and odor, and can even degrade residual disinfectants, further compromising water safety (17). Among the various environmental reservoirs, hospital effluents, untreated domestic wastewater, and groundwater sources used for drinking and irrigation pose the highest human health risks. These reservoirs act as major convergence points for antibiotic residues and resistant pathogens, facilitating direct human exposure through water consumption or indirect transmission via agriculture and food chains. In contrast, surface water and sediments, while significant, represent secondary reservoirs that sustain environmental persistence (21, 26).

While clinical settings are often the primary focus of discussions on antimicrobial resistance, evidence clearly indicates that environmental biofilms, particularly those in water sources in Nigeria, are not merely passive contaminants but active reservoirs for antibiotic-resistant bacteria and their associated resistance genes (16). This implies that even if clinical infections are successfully treated, continuous exposure to AMR from environmental sources can lead to re-infections or the acquisition of new resistance traits, thereby perpetuating a cycle of resistance. This silent dissemination pathway represents a critical public health challenge, especially in LMICs, where water infrastructure and sanitation are frequently inadequate.

Role of Environmental Factors in Biofilm Persistence and AMR Dissemination

Environmental factors play a crucial role in the persistence of biofilms and the dissemination of antimicrobial resistance. The presence of low concentrations of antibiotics in environmental reservoirs, often stemming from agricultural runoff and inadequate wastewater treatment, can exert selective pressure on microbial communities. This constant exposure, even at sub-inhibitory levels, contributes significantly to the development and maintenance of

antibiotic resistance within environmental biofilms (15, 27). The complete set of resistance-related genes in a particular system, termed the environmental resistome, is shaped by such exposures (27).

Furthermore, the virome (the collective viral particles within biofilms, particularly bacteriophages) may contribute to the spread of antibiotic resistance genes through transduction, although this area warrants more extensive investigation to fully understand its ecological implications (11, 27). The widespread distribution and persistence of bacteria like *Staphylococci* in natural environments are largely attributable to their inherent ability to form robust biofilms and tolerate harsh conditions, including dehydration and low water activity (21).

The observation that agricultural practices are major sources of antibiotic resistance transmission to soil and water resistomes, even at sub-inhibitory concentrations, combined with the role of environmental biofilms as resistance reservoirs, establishes a critical and often overlooked causal link between agricultural antibiotic use and clinical AMR. In Nigeria, where agriculture is a dominant economic sector and water treatment infrastructure is limited, this means that practices seemingly far removed from hospitals directly contribute to the burden of untreatable infections in patients. These interconnected drivers, ranging from spanning agriculture, water management and environmental microbiology, underscore the necessity of a unified surveillance system that integrates environmental monitoring with clinical and veterinary data.

Interplay Between Human, Animal, and Environmental Health: The "One Health" Approach

The antimicrobial resistance crisis in Nigeria is particularly severe due to the extensive use of antibiotics across human, animal, and agricultural sectors, compounded by ineffective antimicrobial stewardship programs (14). To effectively mitigate this multifaceted crisis, the adoption of a comprehensive "One Health" approach is indispensable. This framework emphasizes collaborative efforts among governmental agencies, healthcare institutions, veterinary experts, farmers, and the broader scientific community, recognizing the intricate convergence of human, animal, and environmental health (14). Such an integrated strategy is crucial for understanding and addressing the complex dynamics of AMR.

Climate variability, manifested through droughts, floods, and erratic rainfall, further undermines Nigeria's water security (27). Such shifts alter temperature, nutrient load, and pH, creating conditions that favor biofilm formation and persistence in environmental reservoirs (21). These effects heighten

waterborne-disease outbreaks and accelerate AMR spread, making climate change a critical amplifying factor in Nigeria's biofilm-related public-health burden. The interplay between clinical, environmental, and animal health domains underscores the necessity of an integrated One Health framework in addressing

biofilm-associated infections. Figure 4 illustrates this interconnected system, showing how biofilms formed in clinical settings, environmental reservoirs, and agricultural systems interact to sustain antimicrobial resistance and perpetuate reinfection cycles in Nigeria.

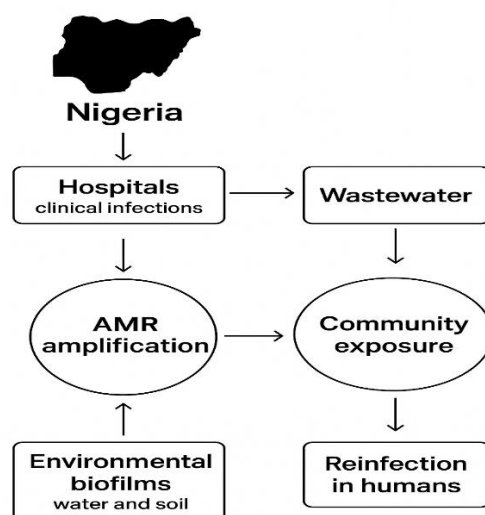


Figure 4: Conceptual link between clinical, environmental, and One Health dimensions of biofilm-associated AMR in Nigeria. Arrows indicate transmission pathways and feedback loops between healthcare facilities, environmental reservoirs, and community interfaces that amplify antimicrobial resistance.

Low-Cost Strategies and Innovative Opportunities for Biofilm Control in LMICs

Addressing the pervasive challenges posed by biofilms in resource-limited settings like Nigeria necessitates the development and implementation of practical, affordable, and accessible interventions. This section explores several promising strategies that align with the need for low-cost solutions.

Experimental and In-Vitro Leads

Research in developing countries is increasingly focusing on the exploration of anti-infective compounds derived from plant sources as a means to control biofilm-induced infections (22). For instance, studies have demonstrated the inhibitory effects of *Macrosphyra longistyla* extracts and Neem (*Azadirachta indica*) leaf essential oil against antibiotic-resistant biofilm-producing bacteria prevalent in Nigeria (28). A particularly promising avenue involves synergistic approaches, where plant extracts are combined with nanoparticles, such as titanium ferrite, to achieve enhanced bioactivity at lower concentrations (22, 29). Furthermore, fungal metabolites enriched with bioactive compounds have shown efficacy in inhibiting multiple factors crucial for biofilm formation, including surface adhesion and cell-to-cell communication through quorum quenching (30).

Nigeria, like many LMICs, possesses rich biodiversity and a long history of traditional medicine. The documented efficacy of local plant extracts and

fungal metabolites against biofilms represents a significant opportunity to develop low-cost, locally sourced antibiofilm agents. This approach reduces reliance on expensive imported pharmaceuticals (31), fostering local research, production, and ultimately contributing to self-sufficiency in combating biofilm infections (29). Such strategies offer sustainable and culturally appropriate healthcare solutions.

Beyond natural products, the repurposing of existing resources and technologies also presents an innovative pathway for LMICs. This includes exploring molecular methods of biofilm dispersal, such as the use of enzymes (proteases, deoxyribonucleases, glycoside hydrolases), antibiofilm peptides, and quorum-sensing inhibitors (30). Although some of these approaches, such as non-thermal plasma therapy, are still technologically advanced, their underlying biological principles can inspire the discovery of affordable local analogues. For example, locally sourced enzymes or plant-derived compounds with comparable antibiofilm activities could be identified and optimized for clinical or environmental applications (32).

While these experimental and laboratory-based innovations hold strong potential, most remain in the preclinical stage, emphasizing the urgent need for translational research and feasibility testing under Nigerian healthcare and environmental conditions.

Deployable and Feasible Interventions

Given the critical role of waterborne biofilms in AMR dissemination and the challenges associated with centralized, high-cost water treatment infrastructure in Nigeria, the development of decentralized and low-cost biofilm management solutions offers a transformative opportunity. These community-scale systems can empower rural and peri-urban populations to locally manage water quality, reducing exposure to pathogenic and antibiotic-resistant biofilms while enhancing public health resilience.

Biofilm technologies such as simple filter systems using readily available materials (e.g., pozzolan and sawdust) have demonstrated high efficiency in removing chemical oxygen demand (COD) and biological oxygen demand (BOD) from wastewater (26). More advanced but still affordable systems, such as moving-bed biological reactors (MBBR) and highly packed biofilm reactors (HPBR), show promise for treating septic tank effluent and rural wastewater with low maintenance requirements (26). Importantly, these systems can harness renewable energy sources like solar and wind power, both abundant in Nigeria, to further minimize operational costs (26). Crucially, these technologies can leverage abundant renewable energy sources, such as solar and wind power, prevalent in many developing countries, to stimulate their treatment processes, further reducing operational expenses (26).

While decentralized solar-powered chlorination and biosand filters have shown promise in other LMICs, evidence of their large-scale implementation in Nigeria remains limited. Adaptation should therefore prioritize pilot testing, local co-design, and integration with Nigeria's water and sanitation agencies to ensure long-term feasibility.

In parallel, effective biofilm control relies on robust community-based public health interventions. Emphasizing preventive measures such as point-of-use water treatment, proper storage practices, and hand hygiene remains essential (16, 24). Addressing barriers to infection prevention and control (IPC), including the availability of personal protective equipment (PPE) and a consistent supply of hygiene materials, is equally important (9, 31). While awareness of hygiene is relatively common, knowledge of biofilm risks and AMR linkage is low among the general population and even among healthcare workers. Hence, culturally sensitive educational campaigns must be coupled with practical training programs to translate scientific understanding into tangible behavioral change.

The feasibility of these interventions depends on local sourcing of materials, training of personnel, and alignment with existing WASH infrastructure. Behavioral change initiatives must also be complemented by material support; awareness alone is insufficient without access to safe water, protective equipment, and essential supplies.

Conclusion

Biofilms represent a significant and amplified threat in Nigeria, a consequence of their inherent resistance mechanisms converging with systemic healthcare resource limitations and a high burden of infectious diseases. The problem extends beyond clinical settings, permeating environmental reservoirs and necessitating a holistic "One Health" approach to achieve effective control. For sustainable impact, low-cost, context-specific solutions are not merely desirable but essential.

Future Research Directions and Policy Recommendations

To address the multifaceted challenges posed by biofilms in Nigeria, a concerted effort in both research and policy is required:

- **Future Research:** Prioritize investigations into Nigeria's indigenous flora to identify novel antibiofilm compounds and elucidate their mechanisms of action. Develop and validate low-cost, rapid diagnostic tools for biofilm infections that are suitable for resource-limited settings. Conduct studies to understand the specific physiological adaptations of biofilms to local environmental

stressors and sub-inhibitory concentrations of antibiotics. Explore the potential of phage therapy and other non-antibiotic strategies as affordable alternatives to conventional antimicrobial treatments. Initiate interdisciplinary studies to assess the impact of climate change on biofilm epidemiology and antimicrobial resistance dissemination in Nigeria. Crucially, focus on translational research to bridge the gap between laboratory discoveries and practical, community-level interventions.

- **Fostering Interdisciplinary Research and Local Innovation:** We recommend the establishment of joint, interdepartmental student research grants and collaborative projects involving departments such as Microbiology, Chemical and Environmental Engineering, and Public Health across Nigerian universities. This cross-sectoral approach should emphasize the discovery and validation of innovative, low-cost antibiofilm technologies that are practical and scalable within local contexts. Key research areas should include: (1) the development of phytochemical-based antibiofilm agents, leveraging Nigeria's rich biodiversity to screen and validate

plant-derived extracts and purified compounds for their ability to disrupt biofilm formation or degrade mature biofilms; (2) the creation of decentralized diagnostic and monitoring tools, focusing on simple, affordable, and rapid biofilm detection kits suitable for both clinical (e.g., wound care) and environmental (e.g., water source) applications to enable timely and targeted interventions; and (3) engineering-based solutions, exploring novel surface coatings, basic flow dynamics, and locally sourced materials to mitigate biofilm growth in healthcare and water distribution infrastructures. This initiative will not only generate locally relevant data and technologies but also cultivate a skilled, interdisciplinary workforce fluent in the “One Health” approach necessary to address the growing biofilm challenge.

- **Integrating Biofilm and AMR Awareness into Grassroots Education:** To ensure long-term and sustainable behavioral change in curbing the drivers of antimicrobial resistance (AMR), it is essential to integrate simplified content on biofilms, microbial hygiene, and responsible antibiotic use into foundational education and community programs. This can be achieved through both curriculum adaptation and community literacy initiatives. At the secondary school level, basic concepts of microbial biofilms (such as their connection to persistent infections and water contamination) should be incorporated into existing science subjects like Biology, Chemistry, and Health Science. Introducing Infection Prevention and Control (IPC) principles at this stage helps students develop an early

understanding of microbial hygiene and the consequences of antibiotic misuse. Complementing this effort, community literacy programs should be designed to deliver simplified and culturally appropriate educational materials that clearly explain the relationship between poor water and sanitation, biofilm reservoirs, and antibiotic misuse. By fostering awareness at both the school and community levels, this integrated approach strengthens the foundation for community-based public health interventions, aligning with the “One Health” framework to promote lasting AMR prevention from the grassroots.

- **Policy Recommendations:** Advocate for increased governmental and private investment in healthcare and medical research in Nigeria, specifically targeting biofilm-related challenges. Implement robust "One Health" policies to regulate antibiotic use across human, animal, and agricultural sectors, and establish comprehensive surveillance programs for environmental antimicrobial resistance. Strengthen infection prevention and control (IPC) programs in healthcare facilities, ensuring the adequate supply of materials and continuous training for healthcare professionals. Promote the adoption of low-cost, decentralized water treatment technologies in rural and underserved urban areas to improve public health outcomes. Integrate public health education campaigns to raise awareness about biofilms, emphasize the importance of hygiene, and promote responsible antibiotic use among the general populace.

Table 4: Recommended Actions and Implementation Framework

Timeframe	Key Actions	Responsible Actors
Short-term (0–2 years)	Implement joint AMR–WASH surveillance programs; strengthen infection prevention and control (IPC) training in tertiary hospitals; improve diagnostic capacity for biofilm detection.	NCDC, Federal Ministry of Health, Teaching Hospitals
Medium-term (3–5 years)	Develop a national biofilm research roadmap; incentivize plant-based compound screening and low-cost intervention trials; enhance intersectoral collaboration among research institutions.	Ministry of Health, NCDC, Universities, Research Institutes
Long-term (5+ years)	Establish a <i>One Health Biofilm Innovation Centre</i> ; integrate biofilm monitoring into national AMR surveillance plans;	Federal Government of Nigeria, Ministry of Environment, International Partners (WHO, CDC, UNEP)

	promote sustainable partnerships with international organizations for technical and financial support.	
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The Nigeria Centre for Disease Control and Prevention (NCDC) and the African CDC can play pivotal roles in scaling joint AMR–WASH surveillance and advancing integrated One Health initiatives. Embedding these interventions within national AMR and water safety action plans will translate research

insights into measurable impact. Implementing these actions could significantly strengthen Nigeria’s resilience against biofilm-associated infections and support global antimicrobial resistance containment efforts.

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References

1. Montanari E, Bernardo G, Le Noci V, Anselmi M, Pupa SM, Tagliabue E, et al. Biofilm formation by the host microbiota: a protective shield against immunity and its implication in cancer. Mol Cancer. 2025;24(1):148. <https://doi.org/10.1186/s12943-025-02348-0>

2. Almatroudi A. Investigating Biofilms: Advanced Methods for Comprehending Microbial Behavior and Antibiotic Resistance. Front Biosci (Landmark Ed). 2024;29:133. <https://doi.org/10.31083/j.fbl2904133>

3. Bayatli N, Malkawi A, Malkawi A, Khaled K, Alrabadi N, Collins Ovenseri A, et al. Impact of biofilms on healthcare settings and management strategies. Rev Med Microbiol. 2024. <https://doi.org/10.1097/MRM.00000000000000425>

4. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. Microorganisms. 2023;11(6):1614. <https://doi.org/10.3390/microorganisms11061614>

5. World Health Organization. Nigeria — WHO Data [Country overview]. 2025 [updated

2025/07/18]. Available from: <https://data.who.int/countries/566>

6. Jonah JH, Samuel G, Abdullahi MI, Emmanuel EA, Danladi SS, Ekwuluo CE. Nigeria's public health response to disease outbreaks: A review of strengths and weaknesses. J Public Health Afr. 2024;15(1):773. <https://doi.org/10.4102/jphia.v15i1.773>

7. Nigeria GML. The State of Healthcare in Nigeria: Challenges and Opportunities. 2025 [updated 2025/07/18]. Available from: <https://gml.com.ng/the-state-of-healthcare-in-nigeria-challenges-and-opportunities/>

8. Umar AA, Salihu HM, Azuine RE. Crisis of Brain Drain in Nigeria’s Health Sector: Challenges, Opportunities, and the Path Forward. Int J MCH AIDS. 14:e011. https://doi.org/10.25259/IJMA_11_2025

9. Ndibuagu EO, Chime OH, Orji CJ, Aneke TJ. Infection Prevention and Control Practices, and Barriers to Compliance among Medical Doctors and Clinical Medical Students, in a State University Teaching Hospital, Southeast Nigeria. J Adv Med Med Res. 2022;34(20):178–

90. <https://doi.org/10.9734/jammr/2022/v34i2031483>
10. Caleb G, Okon A, Inah A, Olanrewaju O, Achi F, Bisongedam M. Practices of Infection Prevention and Control among Primary Healthcare Workers and Associated Factors: A Cross-Sectional Study. Trends Med Res. 2023;18:24-35. <https://doi.org/10.3923/tmr.2023.24.35>
11. Lawal O, Orenolu IO, Ajobiwe MA, Mensah Forson KAA, Agu UF, Fidelix ME, et al. Hospital-Acquired Infections in the Age of Antimicrobial Resistance and Smart Surveillance. Aust J Biomed Res. 2025;1(1):aubm002. <https://doi.org/10.63946/au.biomed/16759>
12. Eze P, Aniebo CL, Ilechukwu S, Lawani LO. Understanding Unmet Healthcare Needs in Nigeria: Implications for Universal Health Coverage. Health Serv Insights. 2025;18:11786329251330032. <https://doi.org/10.1177/11786329251330032>
13. Abbas S, Khan S, Shah M, Aslam J, Nawaz H, Ilyas N, et al. Public Health Threats Posed by Biofilms and Innovative Strategies for their Control. Discoveries. 2024. <https://doi.org/10.15190/d.2024.16>
14. Alabi ED, Rabiou AG, Adesoji AT. A review of antimicrobial resistance challenges in Nigeria: The need for a one health approach. One Health. 2025;20:101053. <https://doi.org/10.1016/j.onehlt.2025.101053>
15. Lawal O. Antibigram and Molecular Characterization of Extended-Spectrum Beta-Lactamase-Producing Klebsiella pneumoniae in a Nigerian Teaching Hospital. Microbes Infect Chemother. 2025. <https://doi.org/10.54034/mic.e2305>
16. Olalemi A, Akinruli F, Oluwasusi V. Antibiotic Resistance Pattern of Bacteria Isolated from Biofilms in Water from Groundwater Sources in Ado-Ekiti, Nigeria. South Asian J Res Microbiol. 2019;1-9. <https://doi.org/10.9734/sajrm/2019/v3i430093>
17. Agbabiaka T, Teslim S, Otuyelu F. Biofilm-forming bacteria and their antibiotic resistance in treated water supplies in Ilorin Metropolis, Nigeria. Ceylon J Sci. 2021;50:199. <https://doi.org/10.4038/cjs.v50i2.7883>
18. Yaki LM, Nwabuisi C, Dagang W, Huyop F. Biofilm Associated Uropathogenic Escherichia coli from Catheterised Patients at a Nigerian Hospital. 2024. <https://doi.org/10.21203/rs.3.rs-4007279/v1>
19. Umar K, Abdullahi IN, Magashi AM, Kawo AH, Usman Y, El-Fulaty Ahmad A, et al. Prevalence and clonal lineages of biofilm-producing Staphylococcus aureus from clinical samples and healthcare workers at Ahmadu Bello University Teaching Hospital, Nigeria. GMS Hyg Infect Control. 2024;19:Doc49. <https://doi.org/10.3205/dgkh000504>
20. Taner F, Baddal B, Theodoridis L, Petrovski S. Biofilm Production in Intensive Care Units: Challenges and Implications. Pathogens. 2024;13(11):954. <https://doi.org/10.3390/pathogens13110954>
21. Silva V, Pereira JE, Maltez L, Poeta P, Igrejas G. Influence of Environmental Factors on Biofilm Formation of Staphylococci Isolated from Wastewater and Surface Water. Pathogens. 2022;11(10). <https://doi.org/10.3390/pathogens11101069>
22. Nwankwo CE, Osho A, Adewuy A, Otuechere C, Olawoye IB, Fayemi SO, et al. Combating oral biofilms in Nigerian schoolchildren: a synergistic approach using Macrospyrha longistyla extracts and titanium-ferrite nanoparticles. GMS Hyg Infect Control. 2025;20:Doc22. <https://doi.org/10.3205/dgkh000551>
23. Mgbodi GO. Inadequate healthcare service administration and management in Nigeria and solutions. 2023. Available from: https://www.researchgate.net/publication/372769855_INADEQUATE_HEALTHCARE_SERVICE_ADMINISTRATION_AND_MANAGEMENT_IN_NIGERIA_AND_SOLUTIONS
24. Adedokun AI, Gana SE, Adenrele BO. Bacteria biofilms: Navigating the way forward. Microbes Infect Dis. 2023. <https://doi.org/10.21608/mid.2023.236659.1621>

25. Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, et al. Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. *Front Microbiol.* 2020;11. <https://doi.org/10.3389/fmicb.2020.00928>
26. Shareef N. Wastewater Treatment by using Biofilm Technologies as a Low-cost Solution for Rural Areas. In: Bui XN, editor. *Biomedical Engineering*. Cham: Springer International Publishing; 2020. p. 1-13. <https://doi.org/10.1007/978-92-022-8701-3>
27. Flores-Vargas G, Bergsveinson J, Lawrence JR, Korber DR. Environmental Biofilms as Reservoirs for Antimicrobial Resistance. *Front Microbiol.* 2021;12. <https://doi.org/10.3389/fmicb.2021.766242>
28. Omoya FO, Salami FF, Ajayi KO. *Azadirachta indica* Leaf Essential Oil as Control of Biofilm-Producing Microorganisms from Hospital Environment Nigeria. *SustainE*; 2025. Available from: <https://sustaine.org/azadirachta-indica-leaf-essential-oil-as-control-of-biofilm-producing-microorganisms-from-hospital-environment/>
29. Oluwole OM. BIOFILM: FORMATION AND NATURAL PRODUCTS' APPROACH TO CONTROL - A REVIEW. *Afr J Infect Dis.* 2022;16(2 Suppl):59-71. <https://doi.org/10.21010/Ajid.v16i2S.7>
30. Ghosh S, Nag M, Lahiri D, Sarkar T, Pati S, Joshi S, et al. New holistic approach for the management of biofilm-associated infections by myco-metabolites. *J Basic Microbiol.* 2022;62(11):1291-306. <https://doi.org/10.1002/jobm.202200047>
31. Srivastava S, Roddam S, Srivastava D, Shehu A, Gogineni KK, Singla R, et al. Exploring Non-antibiotic Interventions for Preventing Urinary Tract Infections During Pregnancy: A Systematic Review. *Cureus.* 2025;17(10):e93874. <https://doi.org/10.7759/cureus.93874>
32. Fleming D, Rumbaugh KP. Approaches to Dispersing Medical Biofilms. *Microorganisms.* 2017;5(2):15. <https://doi.org/10.3390/microorganisms5020015>

Review Article

Evaluating the Role and Impact of International Collaboration and Partnerships on the Control, Prevention, and Elimination of Parasitic Diseases: A Global Health Perspective

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Abstract:

Parasitic diseases continue to represent a significant public health challenge, particularly in tropical and subtropical regions, where they disproportionately affect low- and middle-income countries. Despite significant advancements in global collaborations, diseases such as malaria, schistosomiasis, and leishmaniasis continue to present substantial challenges. This study evaluates the role of international collaborations such as the World Health Organization (WHO) and the Global Fund, alongside bilateral and multilateral initiatives, including the U.S. President's Malaria Initiative (PMI) in managing, preventing, and eliminating parasitic diseases, highlighting the successes, challenges, and potential areas for improvement within these partnerships. Critical challenges to effective collaborations, such as political instability, economic disparities, cultural barriers, and resource inequities were identified. Additionally discussed are the necessity of developing local skills, promoting innovative financing methods for projects, and ensuring their sustainability. Moreover, the necessity of a unified global health governance framework and sustained commitments to ensure that multinational collaborations yield enduring impacts were emphasized. However, policy recommendations such as prioritizing equity, and addressing the root causes of parasite infections aimed at enhancing international cooperation should be embraced in order to achieve sustainable health improvements. The findings suggest that ongoing global cooperation, combined with improvements in technology, funding, and resource allocation, is crucial for the total elimination of parasitic diseases and the advancement of health equity worldwide.

Keywords: Parasitic Diseases; Global Health; Public Health; International Collaboration; Infectious Diseases; Disease Control; Health Equity; Neglected Tropical Diseases

Introduction

A significant number of individuals globally suffer from parasitic diseases, particularly in tropical and subtropical regions. Helminths, ectoparasites, and protozoan parasites are responsible for various parasitic diseases that negatively impact public health, societal well-being, and economic stability. Malaria, schistosomiasis, leishmaniasis, and lymphatic filariasis represent the most prevalent parasitic diseases, contributing significantly to morbidity and mortality rates [1, 2]. Annually, there are over 200 million reported cases of malaria, resulting in more than 400,000 deaths globally [3, 4]. The impact of these diseases imposes considerable strain on constrained healthcare resources, intensifies poverty, and obstructs socioeconomic progress in the communities that are affected.

Effective management of parasite infections necessitates international collaboration. Parasitic diseases pose complex challenges that require global collaboration for effective prevention, management, and sustainable health outcomes. The contributions of NGOs, along with bilateral and multilateral alliances and international health organizations, have led to the successful implementation of significant interventions.

Methodology

This study employed a narrative review approach to evaluate the role and impact of international collaboration and partnerships on the control, prevention, and elimination of parasitic diseases. A comprehensive literature search was conducted (on September 5, 2025) in PubMed and Google Scholar databases to retrieve peer-reviewed research articles relevant to this study. Moreover, grey literature search was conducted by searching directly in Google search engines as well as different reputable organizations databases to retrieve reports, and documents from international organizations such as the World Health Organization (WHO), Centers for

The reduction of malaria can be attributed to the substantial efforts of the Global Fund to Fight AIDS, Tuberculosis, and Malaria, highlighting the importance of international collaboration in this endeavor [5]. The global initiative led by the World Health Organisation (WHO) to eradicate Lymphatic Filariasis serves as a clear illustration of the effectiveness of international collaboration in the fight against parasitic infections [6]. Despite attempts to address and control parasitic diseases, obstacles like political instabilities, economic and infrastructural factors, restricted healthcare access, inadequate diagnostic tools, and treatment resistance persist and impede progress across multiple sectors.

This study aimed to examine global collaboration and partnerships in the prevention, control, and eradication of parasitic diseases. It analyzes the successes and challenges of existing partnerships and programs while proposing improvements in coordination, resource mobilization, and local capacity to strengthen these collaborations. Considering the worldwide scope of these diseases, it is crucial for governments, businesses, and international partners to integrate their resources, expertise, and political will.

Disease Control and Prevention (CDC), and The Global Fund. The search terms included “parasitic diseases”, “international collaboration”, “global health initiatives”, and “disease control” The search focused on articles published in English, with a particular emphasis on studies from the past decade to ensure relevance to current global health initiatives. The narrative synthesis approach was used to integrate findings from various studies and provide an overview of the role of international collaboration in managing parasitic diseases. Data extraction focused on the types of collaborations, their impact on disease control, and the challenges faced by these initiatives.

Global Burden of Parasitic Diseases

Numerous studies indicate that parasitic infections impact factors that extend beyond the health of individuals. Their impact on societies and economies is significant, as they lead to increased healthcare costs, decreased productivity, and the emergence of long-term developmental issues. Developing effective strategies for managing and eradicating parasitic diseases requires a comprehensive understanding of the complexity and scale of the issue at hand [7]. Table 1 illustrates several significant examples that highlight the scale and complexity of this global issue.

Malaria is acknowledged as one of the most serious and lethal parasitic infections. The data indicates that the burden remains significantly high in the WHO African Region, which recorded nearly 263 million cases and 597,000 fatalities in 2023 [4]. This remains accurate despite the evolution of trends over the years. The disease significantly impacts pregnant women and children under five, who represent the highest risk group [4]. The issues of drug resistance and insecticide resistance exacerbate the current situation by diminishing the effectiveness of established control methods, such as bed nets and antimalarial medications

[4]. The battle against malaria illustrates that, despite the implementation of effective techniques, variations in biological and environmental factors can impede advancement. This illustrates the significance of maintaining vigilance and the capacity for adaptation.

Other parasitic diseases significantly affect millions globally. Schistosomiasis, commonly referred to as bilharzia, affects over 240 million individuals worldwide, primarily in Africa, as well as prevalent regions of South America and Southeast Asia [8]. Bowers et al. (2025) [9] and Rinaldo et al. (2021) [10] highlight that schistosomiasis frequently leads to long-term health consequences, such as organ damage and infertility, which can profoundly influence socioeconomic conditions over time. The examination of schistosomiasis and other parasitic diseases reveals a significant relationship with environmental factors, including climate and human activities, and this influences transmission dynamics [11]. Leishmaniasis is also one of parasitic diseases that can have a high

impact on lives globally. There are between 700,000 and 1 million new cases of leishmaniasis each year and Asia, the Middle East, and Latin America are more affected. Leishmaniasis can present in several forms, ranging from disfiguring cutaneous lesions to the potentially fatal visceral variant [12].

The impact of parasitic diseases goes beyond direct distress on humans, it includes slowing down economic growth by making people less productive as a result of illness. The significant impacts on children, including developmental delays and increased mortality rates, jeopardize the welfare of future generations and perpetuate cycles of poverty [13]. There is a need for effective measures to address these issues and this should include not only effective treatments, disease surveillance and vector control, but also broader socioeconomic initiatives that emphasize sanitation, education, and improved access to healthcare.

Table 1: Global burden of major parasitic diseases

Parasitic Disease	Causative Agent(s)	Global Case Estimates	Most Common Regions Affected
Malaria	<i>Plasmodium</i> species	263 million cases in 2023 [3]	WHO African Region: Accounts for 94% of cases, with a disproportionately high burden [3]. Other regions: Southeast Asia, Eastern Mediterranean, Western Pacific, and the Americas [14].
Soil-transmitted helminth (STH) infections	<i>Ascaris lumbricoides</i> , hookworms, <i>Trichuris trichiura</i>	1.5 billion people are infected worldwide [15]	Sub-Saharan Africa, China, South America, and Asia: Highest prevalence, concentrated in poor and deprived communities [15, 16].
Schistosomiasis (Bilharzia)	<i>Schistosoma</i> species	Affects almost 240 million people worldwide [17]	Africa: Accounts for at least 90% of those requiring treatment, with endemic areas also in the Middle East, the Caribbean, and Brazil [17].
Lymphatic filariasis (Elephantiasis)	<i>Wuchereria bancrofti</i> , <i>Brugia malayi</i> , <i>B. timori</i>	51 million infections in 2018 [18]	Asia, Africa, Western Pacific, parts of the Caribbean and South America: Widely distributed in tropical and subtropical regions [18].
Giardiasis	<i>Giardia lamblia</i> (G. duodenalis)	Affects over 250 million people annually [19, 20].	Worldwide: Most prevalent in tropical and subtropical climates and developing countries with poor water quality [19, 20].
Chagas disease (American trypanosomiasis)	<i>Trypanosoma cruzi</i>	6–7 million people infected worldwide [21].	Latin America: Endemic, but global mobility has spread to Canada, the United States, Europe, and parts of Africa, Asia, and the Middle East [22].

Onchocerciasis (River blindness)	<i>Onchocerca volvulus</i>	249.5 million people require preventive treatment (2023 figures) [23].	Sub-Saharan Africa and Yemen: 99% of cases occur here [23]. Latin America: Smaller endemic foci, mainly along the border of Brazil and Venezuela [23].
Leishmaniasis	<i>Leishmania</i> species	An estimated 700,000 to 1 million new cases occur annually [12].	Worldwide: Found on every continent except Australia, with major hotspots in East Africa, the Indian subcontinent, and Brazil [24].
African trypanosomiasis (Sleeping sickness)	<i>Trypanosoma</i> parasites	Cases have fallen to historic low levels, with less than 1,000 cases reported in recent years [25].	Sub-Saharan Africa: Limited to specific endemic foci, with the <i>gambiense</i> form in west and central Africa and the <i>rhodesiense</i> form in east and southern Africa [25].

[This table provides an overview of the global burden of major parasitic diseases, including the number of cases, deaths, and regions most affected. The data highlights the significant impact of these diseases on public health and the need for sustained global efforts to control and eliminate them.]

Role of International Collaboration and Partnerships

The fight against parasitic diseases requires a comprehensive, coordinated approach that leverages the resources, expertise, and political will of various international organizations and governments. This section highlights the contributions and roles of key international health organizations and partnerships as shown in Figure 1, which have played crucial roles in combating parasitic infections.

World Health Organization (WHO)

The World Health Organization (WHO) is a central player in global health efforts, providing leadership, technical expertise, and setting international health standards. Through its various programs, such as the Global Malaria Program and the Department of Control of Neglected Tropical Diseases (NTDs), WHO has been instrumental in shaping global strategies for controlling and eliminating parasitic diseases [26, 27]. WHO coordinates international efforts, sets health priorities, and assists countries in developing policies tailored to local needs. The WHO's commitment to eliminating diseases like lymphatic filariasis, schistosomiasis, and malaria has led to the development of globally accepted guidelines for treatment and prevention [28]. Additionally, WHO supports surveillance, monitors disease trends, and ensures that global health responses are aligned with the most current research and technological innovations [29].

Centers for Disease Control and Prevention (CDC)

The Centers for Disease Control and Prevention (CDC) plays a significant role in providing technical support, conducting research, and building capacity in regions impacted by parasitic diseases. The CDC works

alongside WHO and local health authorities to conduct epidemiological studies, develop diagnostic tools, and implement effective disease surveillance systems. Additionally, the CDC provides training for healthcare workers in endemic regions and contributes to research on new treatments, vaccines, and vector control strategies. By fostering partnerships with national health ministries, the CDC helps strengthen health systems, ensuring that control programs are sustainable and effective in the long term [30].

President's Malaria Initiative (PMI)

Launched in 2005, the President's Malaria Initiative (PMI) is a key U.S. government initiative aimed at reducing malaria-related morbidity and mortality in sub-Saharan Africa. Through PMI, the United States has provided extensive funding, technical assistance, and resources for malaria prevention and treatment [31]. PMI focuses on scaling up proven interventions such as insecticide-treated bed nets, rapid diagnostic tests, and antimalarial treatments, particularly in countries with high malaria burdens [31]. By working with local governments and international organizations, PMI has been instrumental in reducing malaria cases and deaths in several African countries. Its targeted interventions and commitment to improving local health infrastructure have made a significant impact on malaria control efforts [31].

The Global Fund

The Global Fund is a major international financing institution dedicated to combating three of the world's most devastating infectious diseases, including malaria. Established in 2002, the Global Fund has mobilized billions of dollars to support malaria control programs, particularly in sub-Saharan Africa,

where the burden of the disease is highest. The Global Fund supports the distribution of insecticide-treated bed nets, indoor residual spraying, and the provision of antimalarial drugs, among other interventions. Its model of pooling resources from governments, private sector donors, and philanthropies has been pivotal in ensuring that interventions reach the most vulnerable populations. The Global Fund's innovative financing mechanisms and strong monitoring systems help ensure the accountability and effectiveness of funded programs [32].

Department for International Development (DFID)

The UK's Department for International Development (DFID) has long been a key player in the global health landscape, contributing to efforts aimed at controlling parasitic diseases through both direct funding and technical assistance. DFID has been particularly involved in addressing neglected tropical diseases (NTDs) such as schistosomiasis and lymphatic filariasis [33]. Through programs focused on mass drug administration, DFID has supported large-scale efforts to reduce the prevalence of these diseases in endemic regions. DFID has also partnered with international organizations such as WHO and the Global Fund to ensure that interventions are integrated into broader development goals, including improving sanitation, education, and healthcare infrastructure [33].

Bill & Melinda Gates Foundation

The Bill & Melinda Gates Foundation is one of the world's largest private philanthropic organizations, and its contributions to global health have been transformative. The Gates Foundation has made substantial investments in the development of new drugs, vaccines, and diagnostic tools for parasitic diseases. It has supported the development of the RTS,S malaria vaccine, the world's first malaria vaccine, and continues to fund research into innovative ways to control diseases such as schistosomiasis and leishmaniasis. The foundation also plays a pivotal role in fostering public-private partnerships, leveraging the expertise and resources of the private sector to improve access to medicines and health technologies in resource-poor settings [34].

Other Key Contributors

In addition to these major organizations, numerous NGOs and private sector partners play vital

roles in the global fight against parasitic diseases. Organizations such as Médecins Sans Frontières (MSF), the Malaria Consortium, and PATH have worked in the field to deliver essential treatments and provide healthcare services in hard-to-reach areas. These NGOs often work in close partnership with local governments, WHO, and international donors to ensure that interventions are tailored to the specific needs of the communities they serve. The private sector, including pharmaceutical companies like GlaxoSmithKline and Novartis, also contributes by developing and distributing essential medicines and vaccines for parasitic diseases. Their involvement in global health initiatives helps to ensure that treatments are not only effective but also affordable and accessible.

In addition to multilateral organizations and NGOs, bilateral partnerships between countries have played a key role in the fight against parasitic diseases. For example, the United States has partnered with African countries through PMI, while regional cooperation, such as the African Leaders Malaria Alliance (ALMA), has also proven effective in harmonizing efforts across countries to combat malaria. These bilateral and regional partnerships are essential for ensuring that countries work together to address shared health challenges, such as cross-border disease transmission and the development of drug-resistant parasites [35].

Ultimately, while organizations like the WHO, CDC, and The Global Fund play crucial roles in global health initiatives, each has its strengths and limitations. For instance, the WHO's global mandate and technical expertise enable it to set standards and guidelines for disease control. However, its broad scope can sometimes limit its ability to respond rapidly to emerging health issues. The CDC's strength lies in its research capabilities and technical support, but its influence is often constrained by funding limitations and geopolitical considerations. The Global Fund's innovative financing mechanisms have been pivotal in mobilizing resources for disease control programs, but the reliance on donor funding can create uncertainty and sustainability challenges. A comparative analysis of these organizations highlights the need for coordinated efforts and leveraging each organization's strengths to maximize impact.

Challenges to Effective International Collaboration in Combating Parasitic Diseases

There has been a significant amount of progress made in the fight against parasitic diseases as a result of international cooperation; yet, there are persistent challenging factors affecting control and elimination at different phases of collaboration as highlighted in Table

2. Global health collaborations frequently encounter challenges in the areas of funding, coordination, inequity, political dynamics, and other factors that have the potential to undermine even the most robust initiatives [36]. These issues are not only logistical in

nature; rather, there are those relating to health systems management at various levels.

One of the most significant and ongoing challenges is the presence of political instability and shifting priorities. The presence of political instability and corruption might make it challenging for global health projects to be effective in a number of countries particularly the low and middle income countries [37]. For example, the conflict in Sudan has severely impacted malaria control programs, leading to resurgence of cases and complicating efforts to distribute bed nets and provide treatment [38]. Similarly, economic disparities between countries can affect the sustainability of health initiatives. In response to critical problems such as economic downturns, security threats, or public health emergencies like COVID-19, national priorities regularly shift. This shift in priorities may result in a diminished attention on the management of parasitic diseases [39]. Moreover, there are also cases of geopolitical disputes and disagreements between donor nations or between governments and international organizations and these affect the distribution of resources and misalign health initiatives. This can lead to fragmented health policies that either contradict or fail to align with global objectives [40, 41].

Additionally, there are also economic challenges. A significant number of nations that are afflicted by parasitic diseases face challenges in acquiring the requisite resources that are necessary to carry out and maintain comprehensive interventions. As a result, these nations typically rely on financial support from outside sources [42]. Although funding can be quite substantial in certain areas, it is frequently not dispersed in an equitable manner as certain regions with high incidence may not receive sufficient financial support [43]. Due to the unequal distribution of resources, the disease burden continues to worsen, which in turn hinders efforts to eradication.

Notwithstanding, cultural barriers are one of the challenges that hinder international collaboration. Cultural concepts and practices have a major impact on health behaviors, by either facilitating or impeding the adoption of preventative measures and opinions from collaborators from different cultural backgrounds [44]. For example there are misconceptions regarding the safety or efficiency of interventions such as insecticide-treated nets (ITNs) for malaria [45]. The influence of cultural beliefs on the understanding of disease causation and the significance of traditional medicine, can have a significant impact on the effectiveness of

programs. There are situations in which external collaborators may fail to properly connect with local communities or respect cultural practices, which can result in distrust and a reduction in the efficiency of health programs [46]. A strong emphasis on cultural sensitivity and proactive community engagement is required for effective collaboration. This is necessary in order to guarantee that interventions will be accepted and maintained throughout the course of time.

Despite the availability of effective resources, challenges persist in terms of accessibility and equity. Even in situations where treatments are available, marginalized and isolated communities frequently face major difficulties accessing them. These challenges can include geographical isolation, restricted transportation alternatives, and an inadequate healthcare infrastructure [7]. The uneven allocation of resources, which often favors wealthier regions or countries, exacerbates this issue significantly. This disparity in access and resource allocation undermines the fundamental principle of inclusion and hampers progress in the fight against diseases. [47].

Another recurrent difficulty is the lack of effective coordination among organizations. Many global health organizations that are active in this field—ranging from the World Health Organization and the Global Fund to a large number of non-governmental organizations—frequently operate in a disconnected manner, which leads to initiatives that overlap and miss opportunities for collaboration [40]. When organizations do not have clearly defined responsibilities and integrated plans, they run the risk of channeling resources to already addressed issues and pushing agendas that are in contradiction with one another [48].

Moreover, ensuring the sustained viability of programs presents a significant challenge that needs to be addressed. Numerous projects are dependent on short-term financial support from external donors; nevertheless, when this support is terminated or when donor priorities shift, it is possible that programs will not be able to continue operating [37]. For example, the failure of the Global Malaria Eradication Program (GMEP) during the 1950s and 1960s [49]. There were similar challenges that were encountered in the Onchocerciasis Control Program in a number of different countries of West Africa including sporadic funding and weak coordination [50]. Therefore, to ensure continuity in progress, countries should take charge by investing and developing local ability.

Table 2: Challenges to effective international collaboration

Phase in the collaboration	Description	Specific Challenges/Barriers
Input: Donors & International Partners	The source of funding and expertise, involving global organizations (WHO, CDC), multilateral funds (The Global Fund), bilateral agencies, and NGOs.	Unstable Funding: Insufficient or unreliable financial commitments from donors, which can delay or halt long-term programs.
Process: Strategy and Planning	The collaborative development of joint strategies, including program design and intervention selection, tailored for endemic regions.	Lack of Coordination: Poor communication and a lack of harmonized strategies among numerous international organizations
Process: Resource Flow	The central pathway represents the movement of funds, technical expertise, and interventions towards affected populations.	Political Instability/Corruption: Government instability or corruption in recipient countries can disrupt the effective channeling of resources.
Process: Intervention Delivery	The stage where planned health interventions are implemented on the ground in endemic communities.	Cultural and Local Context Barriers: Community distrust, resistance to interventions, or misalignment of programs with local practices.
Outcome: Effective Control Efforts	The intended result of collaboration: reduced parasitic disease prevalence and improved health outcomes.	Inequitable Access: The most vulnerable and geographically isolated populations may be left behind, worsening health disparities.
External Factors (Overarching Influences)	External elements that can affect all stages of collaboration	Climate Change: Shifting environmental patterns that alter disease transmission and introduce parasites into new areas. Drug and Insecticide Resistance: The continuous evolution of parasites and vectors that undermines the effectiveness of interventions.

Strategies for Strengthening International Collaboration

International cooperation is crucial for the management, prevention, and eradication of parasitic diseases. Establishing more robust multinational coalitions that promote effective governance, enhance coordination, develop local capacities, and encourage innovative financing for projects is crucial to bolster international collaboration, thereby ensuring the success of initiatives aimed at addressing parasitic diseases.

Governance and Coordination Mechanisms

For multinational collaborations to be successful, there is a need for a strong governance structure and effective coordination at all levels of government. The establishment of effective governance structures is essential due to the extensive occurrence of these diseases and their significant effects on a vast

population. In these structures, defined roles, duties, and protocols for decision-making should be outlined. The creation of mechanisms that are both transparent and accountable, and that are able to promptly address growing difficulties, such as medication resistance and new outbreaks, is necessary for effective governance. The World Health Organization (WHO) and the Global Fund are two examples of multinational organizations that have developed complete coordination systems in order to improve governance. The implementation of these systems guarantees the effective exploitation of resources and makes it easier to coordinate efforts across a variety of sectors [51].

The structures of coordination should be able to facilitate effective collaboration across a variety of fields, including the fields of education, infrastructure,

and health care. A variety of social factors, including poverty, inadequate nutrition, and poor sanitation, significantly influence health outcomes, with parasitic infections being associated with these conditions. Through effective governance and improved coordination, it is possible to formulate policies that are more comprehensive and cohesive, aimed at tackling the root causes of parasite infections and ultimately enhancing health outcomes[52]. The Global Health Sector Strategy for Neglected Tropical Diseases, as outlined by the World Health Organization, highlights the necessity of addressing the issue through a coordinated and multi-sectoral approach. This indicates that enhancements are necessary for health systems alongside initiatives related to water, sanitation, and education [53].

Enhancing Local Capacity While Fostering International Support

Improving the capabilities of local organizations is absolutely necessary in order to enhance international relationships. Despite the fact that global partners contribute financial and technical support, the capacity of local health systems to implement and maintain these programs is a critical factor that greatly influences the success of disease control strategies. One of the most important strategies for increasing the capacity of the local community is to invest in people. Educating healthcare professionals, providing suitable infrastructure, and ensuring that local populations are involved in the design and execution of health initiatives are all necessary steps in this process [54]. It is possible to ensure that interventions are carried out successfully and continue to be beneficial long after the engagement of foreign partners has ended by providing training to local workers on how to recognize, treat, and prevent parasite illnesses.

In addition, it is essential to encourage local leadership in health programs in order to guarantee the long-term viability of efforts. By providing assistance to local governments, non-governmental organizations (NGOs), and community groups, international partners may help these entities cultivate a feeling of ownership and responsibility, which in turn increases the possibility of attaining positive results. The President's Malaria Initiative (PMI) of the United States and similar initiatives highlight the importance of collaboration with local governments to enhance the chances that national health institutions will assume responsibility for malaria control efforts [31]. The collaborations have led to the development of individualized methods that take into account the issues that are specific to the region. These strategies take into account cultural conceptions of health practices as well as geographical

barriers. To ensure that health interventions align with cultural norms and gain broad acceptance, it is crucial to involve community members in the process.

Innovative Financing and Resource Mobilization

In order to ensure the long-term viability and growth of initiatives aimed at preventing parasitic diseases, it is essential to possess techniques that are capable of effectively mobilizing financial resources [55]. Conventional funding sources, such as government assistance and contributions from charitable organizations, frequently fail to meet the ever-increasing requirements of disease management initiatives. This observation holds significant weight, particularly as the population residing in regions affected by the disease continues to rise. The Global Health creative Technology Fund (GHIT Fund) is a good example of how global collaborations should investigate different funding approaches. These mechanisms include public-private partnerships, impact investment, and creative financial techniques. With the goal of advancing health breakthroughs, this effort encourages collaboration between various entities, including foundations, governments, and the corporate sector [56].

The utilization of resources and expertise from the private sector has been shown to be significantly more effective when it is accomplished through collaborative efforts between the public and private sectors. When it comes to the production and distribution of medicines and vaccines that are intended to prevent parasitic illnesses, pharmaceutical corporations play a significant role. When government agencies, non-profit organizations, and private sector businesses work together to develop collaborative efforts, this helps to ensure that treatments continue to be accessible, inexpensive, and provided in an equitable manner. For example, a malaria vaccine (RTS,S/AS01) was developed through a collaborative effort between the European Union, the World Health Organization, and the Bill and Melinda Gates Foundation [57].

In addition, the utilization of local resources through the utilization of novel financial mechanisms such as co-financing partnerships and tailored bonds can provide an additional boost to global health projects. In the context of the financial system, health bonds are a method in which private investors provide the initial capital for health initiatives, with the expectation of getting return based upon the accomplishment of certain health goals. There is a possibility that this strategy will result in increased funding for programs that are focused at eliminating malaria [58]. The techniques of financing that have been discussed have the potential to generate novel routes

for funding, lessen the dependence of disease control programs on donors from the outside, and raise the

level of sustainability that these programs have over the course of time.

Future Directions

The emergence of new trends and the persistence of ongoing issues significantly influence the global control of parasitic diseases. The intricate health risks associated with parasite diseases necessitate that future global collaborations remain adaptable, innovative, and dedicated. In order to combat parasitic illnesses effectively, it is essential for future global health alliances to adapt to emerging technologies, shifts in political landscapes, and the pressing concerns of equality and sustainability. Innovative technologies combined with sustained governmental commitments are poised to address these challenges effectively.

The integration of technology and innovative concepts is a significant trend in global health collaborations. Innovative approaches to diagnosis, data collection, and disease surveillance have the potential to transform our methods for monitoring, regulating, and eliminating parasitic diseases. Mobile technology is enhancing healthcare in remote or underdeveloped areas. By leveraging real-time data from digital platforms, health workers can enhance their ability to monitor outbreaks, assess the progression of diseases, and identify populations at higher risk of illness [59]. AI and machine learning have the potential to enhance the quality of diagnostic tests and improve the accuracy of disease spread predictions, thereby optimizing intervention strategies [60]. Incorporating GIS into disease mapping and surveillance enhances the precision of interventions, particularly for malaria and schistosomiasis, as spatial variables significantly influence disease transmission patterns.

It is essential to establish the necessary infrastructure and resources to facilitate the widespread application of these technologies in global health initiatives. In numerous locations with limited resources, the digital divide presents significant challenges for equitable access to emerging technologies. For technology to achieve its maximum effectiveness, it must be affordable, accessible, and customized to meet the specific requirements of local

communities. To effectively manage its size and complexity, technology must be utilized alongside programs that enhance capacity, educate local health workers, and fortify healthcare systems.

Innovation involves the development of new technologies and the implementation of unique approaches for securing funding and mobilizing resources. Over the past decade, impact investing, social impact bonds, and public-private partnerships have emerged as prominent mechanisms for financing global health programs. These measures can facilitate funding for the prevention of parasitic diseases, regardless of economic conditions. The Global Fund has successfully identified innovative funding strategies, engaged the private sector in global health initiatives, and utilized financial markets to generate revenue [61]. The trajectory of partnerships will hinge on the enhancement of these models and their effectiveness in securing equitable and sustainable funding for marginalized populations.

Considering these developments, it is important to recognize that considerable challenges persist, particularly regarding the attainment of long-term sustainability. Parasitic illnesses disproportionately affect individuals in lower socioeconomic conditions. Addressing health disparities requires a thorough examination of the underlying issues, including poverty, inadequate healthcare, and substandard living conditions. Future policy suggestions must address both the technical methods for disease control and the social determinants that influence health outcomes. Successful partnerships require a strategic integration of disease control efforts with objectives focused on sanitation, access to clean water, and educational initiatives [62, 63]. This approach, engaging individuals from various sectors, seeks to enhance the resilience of parasitic disease prevention programs against external disruptions such as political instability and economic crises. This will contribute to their extended durability.

Policy Recommendations

A vital policy recommendation for enhancing international collaboration in the management of parasitic diseases is the necessity for a more integrated global health governance structure. The WHO and the Global Fund have provided significant assistance; however, the absence of coordination among international organizations has occasionally resulted in

fragmented projects and inefficiencies. A collaborative strategy, where governments, international organizations, NGOs, and the corporate sector align their resources, research, and activities, will significantly enhance the effectiveness of global health interventions. This framework requires well-defined communication channels, common objectives, and

synchronized funding approaches. Moreover, enhancing governance frameworks at the national level would guarantee that interventions are customized to address local requirements and that nations possess the capability to assume responsibility for disease control initiatives [64].

Future international partnerships must prioritize the sustained health and developmental requirements of endemic nations. This approach ensures that health systems are adequately prepared to manage parasitic diseases once external funding ceases.

Conclusion

Parasitic diseases represent a considerable challenge to public health, especially in low- and middle-income nations. Global cooperation is crucial in addressing these diseases, as no nation or organization can confront parasitic diseases alone. International health initiatives, including the WHO, Global Fund, and partnerships between public and private sectors, have been instrumental in the management and eradication of parasitic diseases. Nonetheless, obstacles remain, including those related to politics, economics, culture, equity, access, and ecology. Consequently, it is crucial for the global health community to foster

For health programs to endure, it is essential that they are embraced by the local population. It is essential for countries to prioritize investment in their health systems, as this reflects a collective responsibility for global health outcomes. Collaborations that extend beyond the traditional donor-recipient framework to incorporate elements such as joint research, technology transfer, and information sharing will contribute to the sustainability and enhanced impact of global health initiatives.

ongoing, equitable collaborations that prioritize the management of parasitic diseases and tackle the socio-economic elements that facilitate their proliferation. Strengthening health systems in endemic regions is crucial, and it is important to investigate innovative financial mechanisms to enhance global health initiatives. In summary, tackling parasitic diseases and improving global health requires joint initiatives, financial commitment, and a shared commitment to health equity. Prompt action is crucial, and it is vital for all stakeholders in global health to participate actively.

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References

1. Pisarski K. The global burden of disease of zoonotic parasitic diseases: top 5 contenders for priority consideration. *Trop Med Infect Dis*. 2019;4(1):44. <https://doi.org/10.3390/tropicalme4010044>
2. Centers for Disease Control and Prevention. Parasites. CDC twenty four seven. Saving Lives, Protecting People; 2024 Nov 14. Available from: <https://www.cdc.gov/parasites/about/index.html#:~:text=Parasitic%20Infections,burden%20placed%20on%20entire%20countries>.
3. World Health Organization. Malaria; 2024 Dec 11. Available from: <https://www.who.int/news-room/fact-sheets/detail/malaria>
4. World Health Organization. World Malaria Report 2020: 20 years of global progress & challenges. 2020 Nov 30. Available from: <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020>
5. The Global Fund, Gavi, World Health Organization. Impactful Partnership in Action: Global Fund, Gavi and WHO: The Global Fund; 2024 Dec. Available from: https://www.theglobalfund.org/media/15327/partnership_impactful-partnership-gavi-global-fund-who_report_en.pdf

6. World Health Organization. Global programme to eliminate lymphatic filariasis: progress report, 2023. *Wkly Epidemiol Rec.* 2024;99(40):565-76. Available from: <https://www.who.int/publications/i/item/who-wer-9940-565-576>
7. Isaev V. The Global Burden of Parasitic Diseases: Prevalence, Mortality and Economic Costs. *Bio Med.* 2024;16(12):760. Available from: <https://www.walshmedicalmedia.com/open-access/the-global-burden-of-parasitic-diseases-prevalence-mortality-and-economic-costs.pdf>
8. Abou-El-Naga IF. Demographic, socioeconomic and environmental changes affecting circulation of neglected tropical diseases in Egypt. *Asian Pac J Trop Med.* 2015;8(11):881-8. <https://doi.org/10.1016/j.apjtm.2015.10.015>
9. Bowers SF, Sonnet F, Downs JA. Schistosomiasis: a neglected cause of infertility in females and males. *Curr Opin Infect Dis.* 2025; Publish Ahead of Print. <https://doi.org/10.1097/qco.0000000000001127>
10. Rinaldo D, Perez-Saez J, Vounatsou P, Utzinger J, Arcand JL. The economic impact of schistosomiasis. *Infect Dis Poverty.* 2021;10(1):134. <https://doi.org/10.1186/s40249-021-00919-z>
11. Liang S, Seto EY, Remais JV, Zhong B, Yang C, Hubbard A, et al. Environmental effects on parasitic disease transmission exemplified by schistosomiasis in western China. *Proc Natl Acad Sci U S A.* 2007;104(17):7110-5. <https://doi.org/10.1073/pnas.0701878104>
12. World Health Organization. Leishmaniasis; 2023 Jan 12. Available from: <https://www.who.int/news-room/fact-sheets/detail/leishmaniasis>
13. Andrade MV, Noronha K, Diniz BP, Guedes G, Carvalho LR, Silva VA, et al. The economic burden of malaria: a systematic review. *Malar J.* 2022;21(1):283. <https://doi.org/10.1186/s12936-022-04303-6>
14. World Health Organization. Malaria: World malaria report 2019. Available from: <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2019>
15. World Health Organization. Soil-transmitted helminth infections; 2023 Jan 18. Available from: <https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>
16. Chen J, Gong Y, Chen Q, Li S, Zhou Y. Global burden of soil-transmitted helminth infections, 1990-2021. *Infect Dis Poverty.* 2024;13(05):68-77. <https://doi.org/10.1186/s40249-024-01238-9>
17. World Health Organization. Schistosomiasis; 2023 Feb 1. Available from: <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis>
18. World Health Organization. Lymphatic filariasis; 2025 Jan 5. Available from: <https://www.who.int/health-topics/lymphatic-filariasis>
19. Tawana M, Onyiche TE, Ramatla T, Thekisoe O. A 'One Health' perspective of Africa-wide distribution and prevalence of *Giardia* species in humans, animals and waterbodies: a systematic review and meta-analysis. *Parasitology.* 2023;150(9):769-80. <https://doi.org/10.1017/s0031182023000513>
20. Centers for Disease Control and Prevention. Giardiasis. Saving Lives, Protecting People; 2023 Sep 26. Available from: <https://www.cdc.gov/parasites/giardia/index.html>
21. World Health Organization. Chagas disease (also known as American trypanosomiasis); 2025 Apr 2. Available from: [https://www.who.int/news-room/fact-sheets/detail/chagas-disease-\(american-trypanosomiasis\)](https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis))
22. Centers for Disease Control and Prevention. About Chagas Disease. Saving Lives, Protecting People; 2025 Aug 25. Available from: https://www.cdc.gov/parasites/chagas/gen_info/detailed.html
23. World Health Organization. Onchocerciasis; 2025 Jan 29. Available from: <https://www.who.int/news-room/fact-sheets/detail/onchocerciasis>
24. Inceboz T. Epidemiology and ecology of leishmaniasis. *Curr Top Negl Trop Dis.* 2019;10. <https://doi.org/10.5772/intechopen.86359>

25. World Health Organization. Trypanosomiasis, human African (sleeping sickness); 2023 May 2. Available from: [https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-\(sleeping-sickness\)](https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness))
26. World Health Organization. Defeating neglected tropical diseases through enhanced collaboration; 2024. Available from: <https://www.who.int/teams/control-of-neglected-tropical-diseases/collaboration>
27. World Health Organization. Partnership established to advance WHO's road map for neglected tropical diseases 2021–2030; 2024 Dec 11. Available from: <https://www.who.int/news/item/11-12-2024-partnership-established-to-advance-who-s-road-map-for-neglected-tropical-diseases-2021-2030>
28. World Health Organization. Global programme to eliminate lymphatic filariasis: progress report, 2023. Wkly Epidemiol Rec. 2024;99(40):565–76.
29. World Health Organization. Health emergencies. Available from: <https://www.who.int/our-work/health-emergencies>
30. Centers for Disease Control and Prevention. Global malaria activities. 2024 Apr 04. Available from: <https://www.cdc.gov/malaria/php/public-health-strategy/global-activities.html>
31. U.S. President's Malaria Initiative. 2020 annual report. Washington, D.C.: U.S. Agency for International Development; 2020. Available from: <https://reliefweb.int/report/world/president-s-malaria-initiative-14th-annual-report-congress-april-2020>
32. The Global Fund. About the Global Fund. 2025, September 10. Available from: <https://www.theglobalfund.org/en/about-the-global-fund/#:~:text=Our%20Mission,overcome%20the%20deadliest%20disease%20threats.>
33. Watts C. Neglected tropical diseases: a DFID perspective. PLoS Negl Trop Dis. 2017;11(4):e0005492. <https://doi.org/10.1371/journal.pntd.0005492>
34. Bill & Melinda Gates Foundation. Our role. Available from: <https://www.gatesfoundation.org/about/our-role>
35. Sikaala CH, Dlamini B, Lungu A, Fakudze P, Chisenga M, Siame CL, et al. Malaria elimination and the need for intensive inter-country cooperation: a critical evaluation of regional technical co-operation in Southern Africa. Malar J. 2024;23(1):62. <https://doi.org/10.1186/s12936-024-04891-5>
36. Nit M. The role of international cooperation in controlling infectious diseases. J Infect Dis Med Microbiol. 2024;8(3):203. Available from: <https://www.alliedacademies.org/articles/the-role-of-international-cooperation-in-controlling-infectious-diseases.pdf>
37. Parkhurst J, Ghilardi L, Webster J, Snow RW, Lynch CA. Competing interests, clashing ideas and institutionalizing influence: insights into the political economy of malaria control from seven African countries. Health Policy Plan. 2021;36(1):35-44. <https://doi.org/10.1093/heapol/czaa166>
38. UNICEF. Ongoing conflict disrupts efforts to control malaria and threatens health of millions of children in Sudan. 2025 Apr 25. Available from: <https://www.unicef.org/sudan/press-releases/ongoing-conflict-disrupts-efforts-control-malaria-and-threatens-health-millions>
39. Ung L, Stothard JR, Phalkey R, Azman AS, Chodosh J, Hanage WP, et al. Towards global control of parasitic diseases in the Covid-19 era: One Health and the future of multisectoral global health governance. Adv Parasitol. 2021;114:1-26. <https://doi.org/10.1016/bs.apar.2021.08.007>
40. Kaminsky R, Mäser P. Global impact of parasitic infections and the importance of parasite control. Front Parasitol. 2025;4:1546195. <https://doi.org/10.3389/fpara.2025.1546195>
41. Parker M, Allen T. De-politicizing parasites: reflections on attempts to control the control of neglected tropical diseases. Med Anthropol. 2014;33(3):223-39. <https://doi.org/10.1080/01459740.2013.831414>
42. Brown GW, Rhodes N, Tacheva B, Loewenson R, Shahid M, Poitier F. Challenges in

- international health financing and implications for the new pandemic fund. *Global Health*. 2023;19(1):97. <https://doi.org/10.1186/s12992-023-00999-6>
43. Hanson K, Brikci N, Erlangga D, Alebachew A, De Allegri M, Balabanova D, et al. The Lancet Global Health Commission on financing primary health care: putting people at the centre. *Lancet Glob Health*. 2022;10(5):e715-72. [https://doi.org/10.1016/s2214-109x\(22\)00005-5](https://doi.org/10.1016/s2214-109x(22)00005-5)
 44. Onwuliri CO, Anosike JC, Oguoma C, Onwuliri VA, Nwoke BE, Dozie IN, et al. The Impact of Cultural Behaviours, Local Beliefs, and Practices on Emerging Parasitic Diseases in Tropical Africa. *Negro Educ Rev*. 2005;56(4):311. Available from: <https://eric.ed.gov/?id=EJ751093>
 45. Ajegena BK, Oti VB. The challenges of using insecticides treated nets (ITNs) in curbing malaria in Nigeria: a 2000–2018 systematic review. *J Infect Dis Epidemiol*. 2020;6(4). <https://doi.org/10.23937/2474-3658%2F1510140>
 46. Aubel J, Chibanda D. The neglect of culture in global health research and practice. *BMJ Glob Health*. 2022;7(9). <https://doi.org/10.1136/bmjgh-2022-009914>
 47. Buzeti T, Madureira Lima J, Yang L, Brown C. Leaving no one behind: health equity as a catalyst for the sustainable development goals. *Eur J Public Health*. 2020;30(Supplement_1):i24-7. <https://doi.org/10.1093/eurpub/ckaa033>
 48. Gustavsen K, Sodahlon Y, Bush S. Cross-border collaboration for neglected tropical disease efforts—Lessons learned from onchocerciasis control and elimination in the Mano River Union (West Africa). *Global Health*. 2016;12(1):44. <https://doi.org/10.1186/s12992-016-0185-5>
 49. Nájera JA, González-Silva M, Alonso PL. Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). *PLoS Med*. 2011;8(1):e1000412. <https://doi.org/10.1371/journal.pmed.1000412>
 50. Boussinesq M. Onchocerciasis control: biological research is still needed. *Parasite*. 2008;15(3):510-4. <https://doi.org/10.1051/parasite/2008153510>
 51. The Global Fund. Information Note Malaria: The Global Fund; 2022 Jul 29. Available from: https://www.theglobalfund.org/media/4768/core_malaria_infonote_en.pdf
 52. van Dale D, Lemmens L, Hendriksen M, Savolainen N, Nagy P, Marosi E, et al. Recommendations for effective intersectoral collaboration in health promotion interventions: results from joint action CHRODIS-PLUS work package 5 activities. *Int J Environ Res Public Health*. 2020;17(18):6474. <https://doi.org/10.3390/ijerph17186474>
 53. World Health Organization. Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030: overview. 2020.
 54. Al-Worafi YM. Healthcare Services in Developing Countries. In: *Handbook of medical and health sciences in developing countries: Education, practice, and research*. Cham: Springer International Publishing; 2023. p. 1-21. https://doi.org/10.1007/978-3-030-74786-2_216-1
 55. Le Gargasson JB, Salomé B. The role of innovative financing mechanisms for health. *World Health Report*. 2010. Available from: <https://cdn.who.int/media/docs/default-source/health-financing/technical-briefs-background-papers/innovativebp12final.pdf>
 56. GHIT Fund. Annual report 2025. Available from: https://www.ghitfund.org/assets/othermedia/annual_report_2024_eng.pdf
 57. Laurens MB. RTS, S/AS01 vaccine (Mosquirix™): an overview. *Hum Vaccin Immunother*. 2020;16(3):480-9. <https://doi.org/10.1080/21645515.2019.1669415>
 58. Oroxom R, Glassman A, McDonald L. Structuring and funding development impact bonds for health: nine lessons from Cameroon and beyond. *Center for Global Development*. 2018 Jan 26. Available from: <https://www.cgdev.org/sites/default/file>

- [s/structuring-funding-development-impact-bonds-for-health-nine-lessons.pdf](#)
59. de Souza Rodrigues D. Digital Health Framework to Combating Neglected Tropical Diseases [Doctoral dissertation]. Universidade Federal do Rio de Janeiro; 2024. Available from: https://w1files.solucaoatrio.net.br/atrio/ufrrj-pep_upl//THESIS/10004423/douglas_de_souza_rodrigues_20241016110609186.pdf
60. Srivastava V, Kumar R, Wani MY, Robinson K, Ahmad A. Role of artificial intelligence in early diagnosis and treatment of infectious diseases. *Infect Dis (Lond)*. 2025;57(1):1-26. <https://doi.org/10.1080/23744235.2024.2425712>
61. Danasekaran R. One health: a holistic approach to tackling global health issues. *Indian J Community Med*. 2024;49(2):260-3. https://doi.org/10.4103/ijcm.ijcm_521_23
62. World Health Organization. Water, sanitation and hygiene (WASH). Available from: https://www.who.int/health-topics/water-sanitation-and-hygiene-wash#tab=tab_1
63. Centers for Disease Control and Prevention. Global Water, Sanitation, and Hygiene (WASH). 2025 Jul 29. Available from: <https://www.cdc.gov/global-water-sanitation-hygiene/about/index.html>
64. Peterson EA, Lange JE, Buckley GJ, editors. Investing in global health systems: sustaining gains, transforming lives. Available from: <https://www.kumc.edu/documents/international/eBOOK-%20Investing%20in%20Global%20Health%20System-Sustaining%20Gains%20Transforming%20Lives.pdf>

Review Article

A Systematic Review of Augmented and Virtual Reality for STEM Learning: Engagement, Cognitive Load, and Transfer Outcomes

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Abstract

Immersive technologies such as augmented reality (AR) and virtual reality (VR) are increasingly used in science, technology, engineering, and mathematics (STEM) education, yet their effects on student engagement, cognitive load, and transfer of learning remain fragmented. This systematic review synthesized empirical research on AR and VR in STEM learning environments to examine how these technologies influence behavioral, emotional, and cognitive engagement; intrinsic, extraneous, and germane cognitive load; and near- and far-transfer outcomes. Following PRISMA 2020 guidelines, we searched major education and psychology databases for studies involving AR/VR STEM interventions with quantified engagement, cognitive load, or transfer measures. Twenty studies met the inclusion criteria. Across the sample, immersive technologies consistently enhanced student engagement, particularly by increasing interest, enjoyment, and time-on-task, although effects on deeper cognitive engagement were more variable. Augmented reality frequently reduced extraneous cognitive load by integrating digital information directly into physical learning tasks, whereas fully immersive virtual reality sometimes increased overall mental effort when environments were perceptually rich or navigation demands were high. Transfer of learning outcomes was generally positive but modest: most studies reported gains in near transfer, defined here as applying what was learned to tasks or problems that closely resemble the original learning context, while evidence for far transfer, defined as applying learning to novel, more complex, or substantially different situations, was limited and inconsistently assessed. Taken together, the findings indicate that immersive technologies most reliably improve student engagement and near-transfer learning in STEM when instructional design deliberately manages cognitive load, rather than relying on immersion alone to produce learning gains. These results underscore the importance of aligning immersive features with targeted learning goals and providing structured guidance and reflection to support meaningful transfer of learning.

Keywords: Augmented Reality; Virtual Reality; STEM Education; Cognitive Load; Learning Transfer

Introduction

Immersive technologies have increasingly transformed how science, technology, engineering, and mathematics education is conceptualized and delivered. Augmented reality enables learners to overlay digital content onto physical environments, while virtual reality creates fully simulated environments where learners can manipulate objects and explore phenomena [1]. Both approaches are grounded in embodied cognition and situated learning, which emphasize that knowledge is developed through direct interaction with meaningful contexts [2]. These technologies provide opportunities for visualizing abstract concepts, engaging in problem-solving, and connecting theoretical understanding with experiential practice. This fragmentation creates a critical gap in the literature: we still lack a clear, integrated understanding of how immersive technologies simultaneously shape students' engagement, cognitive load, and transfer of learning in STEM contexts.

Over the last decade, research on immersive learning has expanded rapidly. Meta-analyses consistently show that well-designed immersive interventions can enhance motivation and learning performance in science, technology, engineering, and mathematics [3,4]. Augmented reality has been shown to improve learning outcomes, particularly in science and engineering content [3]. Virtual reality contributes to higher retention and practical skill acquisition, although outcomes vary by instructional design and discipline [4]. Earlier studies indicated that augmented reality increases engagement and interactivity but also identified barriers, including limited teacher training, technical issues, and superficial integration into curricula [5]. Recent studies emphasize that immersive learning requires intentional design rather than reliance on technological novelty [6,7].

Student engagement is central to understanding the educational value of immersive technology. Engagement encompasses behavioral, emotional, and cognitive dimensions that influence persistence and academic achievement [8]. Immersive environments can heighten engagement by making learning interactive and emotionally stimulating, and students often report increased enjoyment, focus, and sense of presence when using these tools [9]. These outcomes align with motivational theories that emphasize autonomy and competence as drivers of intrinsic motivation [10]. However, sustained engagement depends on pedagogical structure. Without effective scaffolding, technological complexity or novelty fatigue can reduce attention and motivation [11].

Cognitive load theory offers a complementary lens for examining how immersive technologies influence learning. Effective learning requires balancing intrinsic, extraneous, and germane cognitive load [12]. Augmented reality and virtual reality can reduce extraneous load when designed to integrate information coherently across formats [13]. For example, augmented reality visualizations can reduce the need for mentally combining multiple information sources [14]. However, highly immersive virtual environments may increase extraneous load when navigation or sensory elements are not directly relevant to learning [2]. Evidence remains mixed on cognitive outcomes, with some studies reporting reduced mental load in augmented reality lessons and others observing increased mental demand in virtual simulations [15]. These differences suggest that cognitive outcomes depend heavily on design quality and learner experience.

Transfer of learning, the ability to apply knowledge or skills to new contexts, is a central goal of science, technology, engineering, and mathematics education. Immersive environments are assumed to support transfer because they situate learning within authentic tasks [16]. For example, virtual chemistry laboratories enable students to practice experiments safely before applying procedures in real laboratories [17]. Despite this potential, most studies examine only near-transfer effects through posttests, while far transfer is rarely assessed [18]. Some evidence further suggests that highly contextualized virtual reality environments may limit far transfer by anchoring learning to specific settings [2]. Determining when immersive environments facilitate transferable learning remains an empirical challenge. Considering engagement, cognitive load, and transfer simultaneously offers a more holistic understanding of immersive learning than treating these constructs separately, because it links how students feel and behave, how much mental effort they expend, and how well their learning carries over to new tasks.

Although research on immersive learning has grown substantially, few reviews have examined engagement, cognitive load, and transfer together. Many studies focus on isolated outcomes, limiting understanding of how these constructs interact during learning [7]. Rapid technological advancement has also outpaced theoretical integration, making it necessary to reassess assumptions about how immersive media function pedagogically. The present systematic review addresses this gap by examining empirical studies published between 2015 and 2025 that implemented augmented reality or virtual reality in science,

technology, engineering, and mathematics education. It investigates three questions: (1) How do immersive interventions influence student engagement compared with traditional instruction? (2) How do these technologies affect cognitive load, and under what conditions do they facilitate or hinder learning? (3) To

what extent do immersive environments support transfer of learning to new contexts? By clarifying how immersive design features relate to engagement, cognitive processing, and transfer, this review moves beyond technological enthusiasm toward evidence-based understanding of immersive learning.

Materials and Methods

This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement to ensure transparent reporting of search, selection, appraisal, and synthesis procedures [24]. The protocol specified the population, interventions, comparators, outcomes, study designs, and time frame a priori. Methodological decisions were informed by prior syntheses on immersive technologies in education and by guidance for narrative synthesis of heterogeneous evidence [2–4].

Eligibility criteria

We included empirical studies that examined augmented reality or virtual reality interventions used for teaching or learning within science, technology, engineering, or mathematics contexts at any educational level. Eligible designs were randomized controlled trials, quasi-experimental studies with or without comparison groups, single-group pretest-posttest studies, and mixed-methods studies that reported quantitative outcomes. Studies were required to report at least one focal outcome related to engagement, cognitive load, or transfer of learning.

Engagement included behavioral, emotional, or cognitive indicators measured using validated questionnaires, observations, log analytics, or performance proxies [5]. Cognitive load referred to subjective or behavioral indices aligned with cognitive load theory, such as mental effort ratings, NASA-TLX, or dual-task measures [6]. Transfer of learning referred to performance on novel tasks or in new contexts, including near transfer to structurally similar problems and far transfer to distinct or real-world tasks. We limited inclusion to peer-reviewed journal articles and peer-reviewed conference proceedings published in English between 2015 and 2025 to capture contemporary augmented reality and virtual reality systems and classroom implementations. We excluded theoretical or opinion pieces, literature reviews, dissertations, book chapters, and studies that used only desktop simulations without augmented reality or virtual reality capabilities. Studies outside science, technology, engineering, or mathematics domains or those that did not assess any focal outcomes were also excluded. Desktop simulations were excluded because they lack key immersive features such as spatial tracking and embodied interaction, and thus differ

pedagogically from AR/VR environments that provide higher levels of presence and situated interaction.

Information sources and search strategy

A comprehensive search was conducted in Web of Science, Scopus, ERIC, and IEEE Xplore. The search strategy combined controlled vocabulary and free-text terms related to immersive technologies, science, technology, engineering, and mathematics education, and the three outcome constructs. Core strings used Boolean operators and proximity operators where supported. An example scaffold was:

- (“augmented reality” OR “virtual reality” OR “mixed reality” OR “XR” OR “head-mounted display” OR “360 video”)
- AND (“STEM” OR “science education” OR “technology education” OR “engineering education” OR “mathematics education”)
- AND (“engagement” OR “motivation” OR “presence” OR “cognitive load” OR “mental effort” OR “transfer of learning” OR “knowledge transfer” OR “skill transfer”).

Search fields included titles, abstracts, and keywords. To reduce retrieval bias, we screened the reference lists of included studies and recent immersive technology reviews [7–9]. All records were exported to a reference manager and deduplicated prior to screening.

Study selection

Screening occurred in two stages. First, two reviewers independently screened titles and abstracts using a calibrated form. Disagreements were resolved by discussion and consensus. Studies meeting inclusion criteria or lacking sufficient detail were retrieved in full. Second, full texts were independently assessed, and specific exclusion reasons were documented. A third reviewer was available to arbitrate when needed. The flow of records is summarized in a PRISMA 2020 diagram that documents records identified, screened, excluded, and retained [1].

Data extraction

A structured extraction form was developed and piloted to ensure consistency across studies. Two

reviewers independently extracted data and reconciled discrepancies by cross-checking the source texts. Extracted information included bibliographic details, country and study setting, participant characteristics and educational level, the specific science, technology, engineering, or mathematics domain addressed, the intervention type and platform, hardware and software specifications, duration and dosage of the intervention, instructional design features, comparison conditions when applicable, research design, sampling approach, and implementation context such as classroom, laboratory, or field environments. For outcomes, we recorded constructs, instruments used to measure them, timing of measurements, and summary statistics including means, standard deviations, test statistics, confidence intervals, and reported effect sizes. Engagement outcomes were categorized as behavioral, emotional, or cognitive. Cognitive load was classified as intrinsic, extraneous, or germane when the study allowed such attribution, and transfer outcomes were coded as either near transfer or far transfer depending on the extent of contextual shift. Additionally, contextual variables such as implementation challenges, teacher preparation, and learner familiarity with augmented or virtual reality tools were captured when reported, given their potential influence on both engagement and cognitive processing [4,10].

Risk of bias and quality appraisal

Methodological quality was appraised using the Mixed Methods Appraisal Tool (MMAT) 2018 [11]. Each study was evaluated based on design-specific criteria including sampling strategy, randomization or allocation procedures when applicable, management of confounding variables, validity and reliability of outcome measures, completeness of outcome data, and consistency between conclusions and evidence. Two reviewers completed MMAT independently and resolved disagreements by discussion. No studies were excluded solely on quality; instead, MMAT ratings informed interpretation, sensitivity to methodological limitations, and weighting of evidence. We also noted whether studies preregistered protocols, reported power analyses, or documented handling of missing data.

Data synthesis

Due to heterogeneity in technologies, educational contexts, research designs, and measurement tools, a narrative synthesis approach was used [4]. Studies were grouped by primary outcome (engagement, cognitive load, transfer of learning) and by technology type (augmented reality or virtual

reality). When multiple studies used comparable measures, we summarized ranges of effects. Design features historically associated with cognitive or motivational mechanisms — such as alignment with multimedia principles, representational appropriateness, degree of immersion, interaction, and scaffolding — were recorded and interpreted using cognitive load and motivation frameworks [6,12,2]. Mixed-methods studies contributed qualitative themes related to learner or teacher perceptions of engagement and cognitive effort. These themes contextualized quantitative findings.

Subgroup and sensitivity considerations

Subgroup analyses considered augmented versus virtual reality, education level (K–12 versus higher education), learner experience level, science or technology subject, task type, and immediate versus delayed outcomes. Sensitivity was assessed by comparing findings from higher-quality studies with those from studies of moderate or lower quality. We noted instances where convenience sampling, small sample sizes, or unvalidated measures might inflate or reduce effects.

Assessment of reporting bias and certainty

Because of heterogeneity in instruments, formal assessments of reporting bias were not feasible. Potential publication bias was considered by examining the proportion of null results and inclusion of conference proceedings [25]. Certainty of evidence was evaluated by weighing the number of studies, consistency of effects, directness of outcomes, and methodological quality. To ensure data reliability, we used a structured coding scheme and had two reviewers independently screen and code a subset of studies, resolving discrepancies through discussion until consensus was reached.

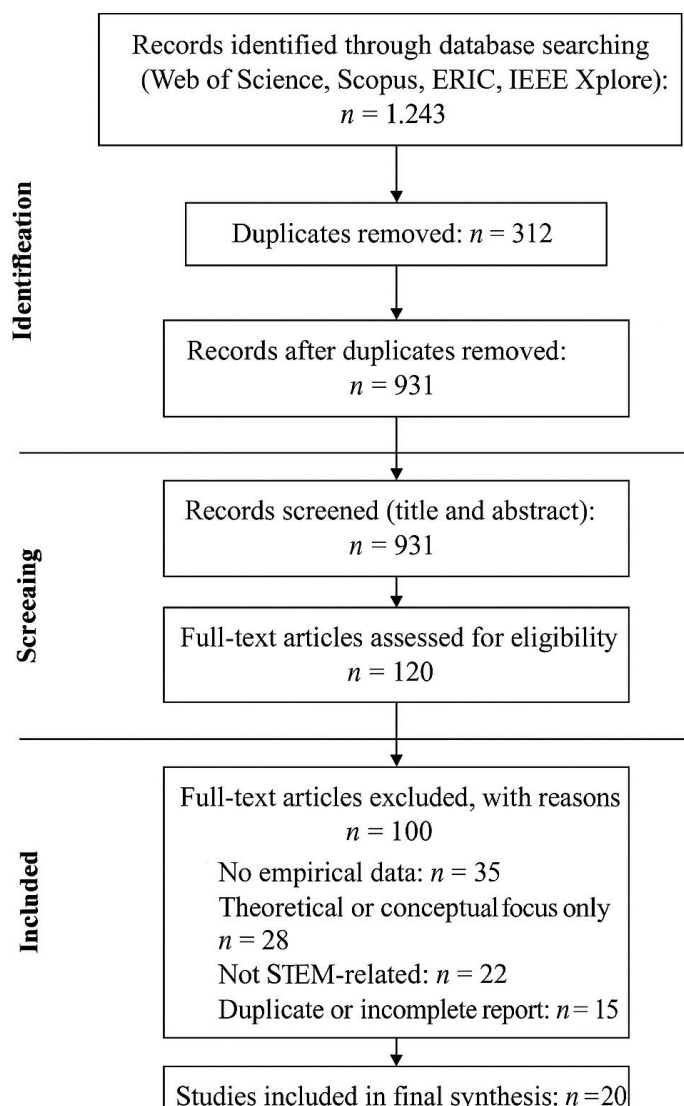
Deviations from protocol

All methodological decisions were defined prior to synthesis. Minor refinements to extraction fields after piloting are noted in the Results section.

PRISMA flow of study selection

The search retrieved 1,243 records. After removing 312 duplicates, 931 were screened by title and abstract. A total of 120 full texts were reviewed, and 100 were excluded due to lack of empirical data ($n = 35$), conceptual focus ($n = 28$), irrelevant discipline ($n = 22$), or methodological issues ($n = 15$). Twenty studies met all inclusion criteria. Twelve examined augmented reality, six examined virtual reality, and two included hybrid approaches [7,13].

Figure 1. PRISMA Flow chart



Results

Overview of included studies

Twenty studies published between 2015 and 2025 met the eligibility criteria. Twelve focused on augmented reality (AR), six examined fully immersive virtual reality (VR), and two compared hybrid AR/VR approaches. As summarized in Table 1, studies were conducted across a range of STEM learning environments including K–12 classrooms, undergraduate courses, and informal or professional development settings in countries such as Spain, the United States, China, Denmark, Turkey, Taiwan, Singapore, Colombia, and Australia. Sample sizes ranged from 30 to 210 participants. Most studies used quasi-experimental or randomized controlled designs and applied validated instruments, including the Intrinsic Motivation Inventory, Paas Mental Effort Scale, NASA-TLX workload index, and standardized learning assessments to measure engagement,

cognitive load, or transfer of learning. Overall methodological quality was rated as high to moderate using the Mixed Methods Appraisal Tool, with most studies demonstrating clear reporting of research designs, appropriate outcome measures, and adequate analytical procedures. Studies involving AR commonly reported increased motivation, engagement, and conceptual understanding, sometimes accompanied by reduced extraneous cognitive load. In contrast, VR studies consistently reported strong engagement effects, with some documenting increases in cognitive load depending on immersion level and user familiarity. Collectively, these findings indicate that immersive technologies are being implemented across diverse educational levels and contexts, using rigorous study designs and validated measurement tools to examine learning outcomes.

Table 1 Characteristics of Included Studies on AR and VR in STEM Education (n = 20)

Author(s) & Year	Country	Education Level	STEM Domain	Technology Type	Sample Size	Design	Instruments Used	Duration	Key Findings / Outcomes	Quality (MMAT)
Ibáñez & Delgado-Kloos [1]	Spain	Undergraduate	Engineering	AR	50	RCT	Motivation Survey, Paas Load Scale	2 weeks	AR enhanced motivation and reduced extraneous load compared with traditional lab	High
Makransky & Mayer [2]	Denmark	High school	Science	VR	100	RCT	IMI, NASA-TLX	3 weeks	VR improved engagement but slightly increased cognitive load; performance unchanged	High
Chang et al. [9]	Taiwan	Secondary	Chemistry	AR	50	RCT	NASA-TLX, Post-test	1 week	AR improved problem-solving and near transfer; mild increase in mental effort	High
Parong & Mayer [18]	USA	Undergraduate	Biology	VR	80	Lab experiment	Interest & CL ratings	Single session	Higher engagement but lower transfer than desktop simulation	High
Mystakidis et al. [6]	Global	Higher ed	Multidomain STEM	AR/VR review	–	Mixed methods	Literature synthesis	–	Identified positive engagement trends; noted gaps in long-term transfer evidence	Moderate
Liu et al. [15]	China	Secondary	Physics	VR	90	Quasi-experimental	NASA-TLX, Achievement test	2 weeks	Immersive VR elevated motivation and germane load; small cognitive overload for novices	High
Cai et al. [13]	Taiwan	Secondary	Mathematics	AR	60	Quasi-experimental	AR attitude scale, learning test	1 week	AR increased learning gains and enjoyment in probability concepts	High
Sirakaya &	Turkey	K–12	Mixed STEM	AR	45	Quasi-experimental	CL survey, observation checklist	2 weeks	AR fostered behavioral engagement;	Moderate

Author(s) & Year	Country	Education Level	STEM Domain	Technology Type	Sample Size	Design	Instruments Used	Duration	Key Findings / Outcomes	Quality (MMAT)
Sirakaya [7]									moderate extraneous load	
Hsu et al. [21]	USA	Elementary	Digital literacy	AR creation	40	Longitudinal	Observation logs	4 weeks	Sustained cognitive engagement over multiple sessions	High
Huang et al. [23]	Taiwan	Secondary	Earth science	AR	58	Quasi-experimental	Motivation & presence scales	1 week	Enhanced situational interest and immersion	Moderate
Klingenberg et al. [27]	Denmark	High school	Physics	VR	45	RCT	NASA-TLX, concept tests	2 weeks	Comparable conceptual understanding between VR and real labs	High
Radianti et al. [4]	Global	Higher ed	Various STEM	VR review	—	Review	Literature screening	—	Synthesized 40 studies; reported strong engagement effects	High
Johnson-Glenberg et al. [20]	USA	Middle	General science	AR	52	Quasi-exp.	Engagement survey, transfer test	2 weeks	AR increased interaction and far transfer performance	Moderate
Akçayır & Akçayır [5]	Turkey	Various	STEM	AR	42	Quasi-exp.	Questionnaire, interviews	1 week	Improved motivation; usability issues raised cognitive demand	Moderate
Garzón [3]	Colombia	Higher ed	STEM meta-analysis	AR	—	Review	Meta-analytic dataset	—	Mean effect $g \approx 0.68$ on learning outcomes	High
Önal & Önal [28]	Turkey	Secondary	Astronomy	AR	48	Quasi-exp.	Interest & test performance	2 weeks	AR improved achievement and interest in astronomy	High
Huang et al. [22]	Taiwan	Elementary	Environmental science	AR	50	Quasi-exp.	Attitude & observation	1 week	Increased curiosity and participation in eco-education	Moderate
Lin et al. [31]	China	Teachers (PD)	STEM integration	AR	36	Quasi-exp.	Observation, reflection logs	3 weeks	AR communities improved instructional design capacity	Moderate

Author(s) & Year	Country	Education Level	STEM Domain	Technology Type	Sample Size	Design	Instruments Used	Duration	Key Findings / Outcomes	Quality (MMAT)
Chng et al. [29]	Singapore	K-12	STEM	AI/VR	60	Review	Thematic synthesis	–	Found synergy between AI and VR for engagement	Moderate
Matovu et al. [33]	Australia	University	Chemistry	VR	75	Mixed methods	Test scores, interviews	3 weeks	VR improved spatial reasoning and satisfaction	High

Effects on Cognitive Load

Table 2 summarizes how cognitive load was measured and interpreted across the included studies. Results were not uniform. Approximately half of the studies reported that augmented reality (AR) reduced extraneous cognitive load by integrating digital visualizations directly into the learning context, which reduced split attention and supported more efficient processing [14][18]. In contrast, fully immersive virtual reality (VR) environments frequently increased mental effort due to interface complexity, sensory richness, and navigation demands [27][32]. Across studies, VR

required more working memory resources, particularly among novice users who needed additional time to orient themselves in the virtual space. AR interventions generally optimized cognitive processing by presenting spatial and textual information within the same visual field, allowing learners to focus on conceptual understanding rather than interface management [14]. However, studies also reported that headset weight, interaction mechanisms, and technical difficulties introduced extra cognitive demand, increasing extraneous load even when the intervention was engaging.

Table 2 Cognitive Load Measures and Findings across AR and VR Studies

Study	Construct Focus	Measurement Tool	Comparison Condition	Key Results	Interpretation
Ibáñez & Delgado-Kloos [1]	Extraneous	Paas Mental Effort	Traditional lab	Lower cognitive load in the AR group	AR reduced split-attention demands
Makransky & Mayer [2]	Intrinsic + Extraneous	NASA-TLX	Desktop simulation	Slightly higher overall load in VR	Realism increased demand but not difficulty
Cai et al. [13]	Germane	Custom CL scale	Conventional class	Moderate productive effort	Constructive interaction increased germane load
Parong & Mayer [18]	Extraneous	Self-rated difficulty	PowerPoint lesson	Higher load in VR	Sensory complexity taxed working memory
Klingenberg et al. [27]	Intrinsic	NASA-TLX	Physical lab	Similar intrinsic load	Cognitive demands are comparable between modalities
Sirakaya & Sirakaya [7]	Extraneous	CL questionnaire	No-AR control	Moderate load	Interface moderately demanding
Chang et al. [9]	Extraneous	NASA-TLX	Textbook	Slight load increase	Novel AR display imposed minimal additional effort
Liu et al. [15]	Germane + Extraneous	NASA-TLX	Control	Mixed results	VR boosted productive focus but fatigued novices

Effects on Transfer of Learning

Table 3 summarizes the transfer outcomes, assessment types, and key findings. Transfer of learning was evaluated in 14 of the 20 included studies.

Most studies reported improvements in near transfer, meaning learners were able to apply newly acquired knowledge or skills to tasks that were structurally similar to the instructional activity [19]. These gains

were particularly evident in augmented reality interventions, where visual and contextual cues supported the connection between abstract concepts and applied problem-solving [16][20]. Evidence for far transfer was more limited. Only a few studies demonstrated that learning transferred to novel or distinct contexts beyond the immediate instructional task. Interventions that paired immersive environments with structured reflection or debriefing activities showed stronger far-transfer performance, indicating that immersion alone is insufficient and that metacognitive guidance strengthens generalization of skills and concepts [15][16].

Table 3. Transfer of Learning Outcomes in AR and VR STEM Studies

Study	Domain	Transfer Type	Assessment Method	Key Results	Interpretation
Chang et al. [9]	Chemistry	Near + Far	Problem-solving task	Improved near and far transfer performance	AR facilitated conceptual application
Johnson-Glenberg et al. [20]	Science	Far	Novel scenario test	Higher far-transfer scores	Embodied AR promoted deep understanding
Parong & Mayer [18]	Biology	Near	Post-lesson application	No significant transfer gain	Cognitive overload limited performance
Klingenberg et al. [27]	Physics	Near	Concept quiz	Comparable to a real lab	Equivalent skill transfer achieved
Cai et al. [13]	Math	Near	Post-test	Higher test accuracy	AR improved spatial reasoning
Liu et al. [15]	Physics	Near	Applied task	Better procedural accuracy	Immersive practice aided near transfer
Ibáñez & Delgado-Kloos [1]	Engineering	Near	Design task	Improved circuit design performance	AR linked theory to real components
Matovu et al. [33]	Chemistry	Far	Qualitative evaluation	Partial far-transfer evidence	Learners applied spatial reasoning to new tasks

Note: collectively, these findings suggest that immersive technologies most reliably enhance near transfer in conceptual and procedural domains, particularly when

tasks mirror authentic contexts. Few studies tested far transfer longitudinally, revealing a gap for future research on durable learning beyond the immediate intervention.

Discussion

This systematic review synthesized twenty empirical studies evaluating augmented and virtual reality in STEM education. The findings show that immersive technologies consistently enhance student engagement, whereas effects on cognitive load and transfer of learning are more variable. These outcomes align with prior reviews that also identified strong motivational benefits of immersive tools and noted that cognitive effects depend on instructional design choices [1,2,5]. Overall, the results indicate that AR and VR achieve their strongest educational impact when aligned with cognitive load theory and motivation frameworks. A consistent pattern was the enhancement of student engagement. Learners demonstrated increased interest, attention, and persistence when using AR or VR, particularly when integrated into

authentic activities that required problem solving [3,10]. Studies using AR to represent mathematical or engineering concepts in real contexts reported sustained behavioral engagement and higher participation [7,8]. VR-based activities elicited strong emotional engagement, with learners describing a sense of presence and immersion that exceeded traditional simulations [4,12]. However, qualitative evidence indicated that engagement driven by novelty may fade over time unless educators scaffold learning to channel initial excitement into continued involvement. This emphasizes the importance of guided instruction to ensure that engagement supports deeper learning.

Cognitive load findings were more complex. Several studies showed that AR reduced extraneous cognitive load by aligning visualizations and text

within the learner's physical environment, preventing split attention [7,8]. This supports multimedia learning theory, which proposes that cognitive efficiency improves when information is spatially and temporally integrated [18]. VR, however, often increased mental effort because of the complexity of head-mounted displays, navigation challenges, and sensory stimulation [4,13]. Learners unfamiliar with immersive interfaces were more vulnerable to overload. Thus, the same features that make VR motivating can also impose unnecessary cognitive demands.

Importantly, increased cognitive load was not always negative. When students had prior experience with immersive tools or received pretraining, higher germane cognitive load supported deeper cognitive processing and problem solving [7,12]. This observation is consistent with the three-component structure of cognitive load theory, which distinguishes extraneous load from intrinsic and germane load. Germane load enhances schema formation when extraneous load is minimized [18,19]. Effective implementation, therefore, requires balancing conceptual challenge with usability so that technical complexity does not distract from core learning objectives. Although increases in cognitive load in fully immersive VR environments may appear problematic, they do not necessarily imply that VR is unsuitable for STEM learning. Rather, they highlight the need for careful instructional design that channels mental effort toward relevant learning processes instead of interface management or sensory novelty. Instructional strategies such as simplifying early VR tasks, scaffolding navigation and controls, segmenting complex experiences into shorter, goal-focused episodes, and providing pre-training on key concepts and interactions can help reduce extraneous load while preserving the sense of presence and immersion. Additionally, aligning visual and interactive elements strictly with core learning objectives, instead of adding unnecessary decorative details, can prevent overload from perceptually rich scenes. In this way, VR-based instruction can maintain high immersion while ensuring that cognitive resources are devoted primarily to germane processing and meaningful learning.

Transfer of learning outcomes further illustrates this balance. Most studies reported gains in near transfer, meaning the ability to apply learning to similar tasks. This was particularly evident in AR

studies where situated visualization appeared to bridge abstract concepts with real-world representations [6,9]. Far transfer, which requires applying learning to new tasks or contexts, was examined less frequently and showed weaker effects. However, studies that incorporated structured debriefing and reflective activities demonstrated stronger far transfer outcomes [11,16]. These findings suggest that immersive technologies alone do not produce conceptual generalization. Transfer is maximized when immersive experiences are followed by guided reflection that helps learners connect experiences to disciplinary principles.

A central insight from this review is the relationship between engagement and cognitive load. High engagement can coexist with elevated cognitive load, especially in VR environments where immersion demands significant attention. The relationship appears nonlinear. Moderate cognitive effort can support deep learning, but excessive load may hinder comprehension despite high motivation. This reflects dual processing models, which propose that optimal learning occurs when learners are emotionally engaged but not cognitively overwhelmed [18,19]. Future research should explore how pacing, scaffolding, and adaptive feedback can help regulate mental effort during immersive learning. The synthesis also reveals methodological progress in the field. Earlier studies often relied on small samples and single-session activities [26, 27]. More recent work demonstrates stronger theoretical grounding, clearer designs, and improved outcome reporting [2,5]. Nonetheless, gaps remain. Long-term outcomes are rarely measured. Implementation costs and teacher training requirements are seldom reported, which limits understanding of scalability. Most studies occurred in well-resourced educational systems, which constrains generalizability to low-resource contexts. Future work should expand research to diverse educational settings, address sustainability and cost issues, and explore teacher capacity building.

Taken together, these results suggest that immersive technologies are most effective in STEM education when they are used not simply to increase engagement, but to deliberately manage cognitive load in ways that support the transfer of learning to new tasks and contexts.

Conclusion

The implications for educational practice are substantive. Teachers and curriculum designers can use augmented and virtual reality to foster curiosity, engagement, and conceptual understanding, provided that implementations follow evidence-based

instructional design principles. In augmented reality settings, effectiveness is maximized when visual overlays directly support instructional goals rather than adding decorative elements that increase cognitive noise [30, 32]. For virtual reality, guided exploration,

user orientation, and controlled pacing are essential to avoid cognitive overload, especially for novice users [18]. Hybrid or mixed-reality environments may also provide a balanced alternative, combining the motivational benefits of immersion with the cognitive manageability of real-world grounding. At the institutional level, adoption should be paired with professional development and ongoing communities of practice to help educators integrate immersive technologies into curriculum planning and assessment processes [33].

At a theoretical level, this review reinforces the growing consensus that immersive technologies influence learning through affective and cognitive pathways rather than solely through technological novelty. By increasing situational interest and supporting embodied interaction, immersive environments create conditions for deeper conceptual engagement [7][12]. At the same time, these affordances require careful design to ensure cognitive load remains within optimal ranges. The findings demonstrate that

the success of AR and VR is determined less by the technology itself and more by how it is structured, scaffolded, and embedded within coherent learning ecosystems [20].

This review has several limitations. Although the search and screening process followed validated systematic review procedures, the synthesis included studies that used heterogeneous measures of engagement, cognitive load, and transfer, limiting the ability to conduct a meta-analysis. Restricting the review to English-language publications may have excluded evidence from rapidly innovating regions, and most studies focused on short-term post-intervention outcomes. Future research should incorporate longitudinal designs, standardized measurement tools, implementation fidelity reporting, and cost-benefit analyses to support scalability. Additional research is also needed in diverse educational contexts, especially in primary education settings and in low-resource environments where immersive technologies remain under-studied.

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Authors Contribution: All authors contributed substantially to the development of this systematic review.

Ogunjobi, Damilare Timothy: Led the conceptualization, search strategy design, data extraction framework, and initial drafting of the manuscript.

Stephen Abu: Contributed to methodological oversight, screening of studies, and critical revisions of the manuscript.

Ademola Busayo Ajayi: Assisted with data extraction, quality appraisal, and interpretation of results.

Maureen Ifeyinwa Emenike: Supported literature screening, synthesis, and editing for theoretical alignment.

Enobong Edoabasi Obong: Served as corresponding author; coordinated team activities, contributed to data interpretation, and reviewed the final manuscript.

Thomas Kofi Mensah: Contributed to data analysis, visualization, and validation of the narrative synthesis. All authors reviewed and approved the final version of the manuscript.

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References

1. Ibáñez M-B, Delgado-Kloos C. Augmented reality for STEM learning: A systematic review. *Comput Educ.* 2018;123:109–23. <https://doi.org/10.1016/j.compedu.2018.04.002>
2. Makransky G, Mayer RE. Benefits of taking a virtual field trip in immersive virtual reality: Evidence for the immersion principle in multimedia learning. *Educ Psychol Rev.* 2022;34(3):1771–98. <https://doi.org/10.1007/s10648-022-09692-2>
3. Garzón J, Acevedo J. Meta-analysis of the impact of augmented reality on students' learning gains. *Educ Res Rev.* 2019;27:244–60. <https://doi.org/10.1016/j.edurev.2019.04.001>
4. Radianti J, Majchrzak TA, Fromm J, Wohlgenannt I. A systematic review of immersive virtual reality applications for higher education. *Comput Educ.* 2020;147:103778. <https://doi.org/10.1016/j.compedu.2019.103778>

- 03778"><https://doi.org/10.1016/j.compedu.2019.103778>
5. Akçayır M, Akçayır G. Advantages and challenges associated with augmented reality for education: A systematic review. *Educ Res Rev.* 2017;20:1–11. <https://doi.org/10.1016/j.edurev.2016.11.002>
6. Mystakidis S, Christopoulos A, Pellas N. A systematic mapping review of augmented reality applications to support STEM learning in higher education. *Educ Inf Technol.* 2022;27:1883–927. <https://doi.org/10.1007/s10639-021-10682-1>
7. Sirakaya M, Sirakaya DA. Augmented reality in STEM education: A systematic review. *Interact Learn Environ.* 2022;30(8):1556–69. <https://doi.org/10.1080/10494820.2019.1635497>
8. Fredricks JA, Blumenfeld PC, Paris AH. School engagement: Potential of the concept, state of the evidence. *Rev Educ Res.* 2004;74(1):59–109. <https://doi.org/10.3102/00346543074001059>
9. Chang R-C, Chung L-Y, Huang Y-M. Interactive augmented reality for plant education: Comparing AR and video. *Interact Learn Environ.* 2016;24(6):1245–64. <https://doi.org/10.1080/10494820.2014.982132>
10. Deci EL, Ryan RM. The “what” and “why” of goal pursuits. *Psychol Inquiry.* 2000;11(4):227–68. https://doi.org/10.1207/S15327965PLI1104_01
11. Beyoğlu D, Hürsen Ç, Nasiboğlu A. Use of mixed reality applications in teaching of science. *Educ Inf Technol.* 2020;25(5):4271–86. <https://doi.org/10.1007/s10639-020-10166-8>
12. Sweller J. Cognitive load during problem solving: Effects on learning. *Cogn Sci.* 1988;12(2):257–85. https://doi.org/10.1207/s15516709cog1202_4
13. Cai S, Liu ER, Shen Y, Liu C-H, Li S-H, Shen Y-H. Probability learning using AR: Impact on gains and attitudes. *Interact Learn Environ.* 2020;28(5):560–73. <https://doi.org/10.1080/10494820.2018.1548499>
14. Liu Q, Yu S, Chen W, Wang Q, Xu S. AR magnetic experimental tool effects on knowledge and cognitive load. *J Comput Assist Learn.* 2021;37(3):645–56. <https://doi.org/10.1111/jcal.12424>
15. Liu R, Wang L, Koszalka TA, Wan K. Effects of immersive VR classrooms on achievement, motivation, and cognitive load. *J Comput Assist Learn.* 2022;38(5):1422–33. <https://doi.org/10.1111/jcal.12682>
16. Markowitz DM, Laha R, Perone BP, Pea RD, Bailenson JN. VR field trips to facilitate learning about climate change. *Front Psychol.* 2018;9:2364. <https://doi.org/10.3389/fpsyg.2018.02364>
17. Tsai C-Y, Ho Y-C, Nisar H. Virtual chemistry laboratories. *Appl Sci.* 2021;11(21):10070. <https://doi.org/10.3390/app112110070>
18. Parong J, Mayer RE. Learning science in immersive virtual reality. *J Educ Psychol.* 2018;110(6):785–97. <https://doi.org/10.1037/edu0000241>
19. Zhang W, Wang Z. Theory and practice of VR/AR in K–12 science. *Sustainability.* 2021;13(22):12646. <https://doi.org/10.3390/su132212646>
20. Johnson-Glenberg MC, Birchfield DA, Tolentino L, Koziupa T. Embodied learning in mixed reality: Two science studies. *J Educ Psychol.* 2014;106(1):86–104. <https://doi.org/10.1037/edu0000241>

- href="https://doi.org/10.1037/a0034008"><https://doi.org/10.1037/a0034008>
21. Hsu H-P, Zou W-T, Hughes JE. Developing digital literacy through AR creation. *J Educ Comput Res.* 2019;57(6):1400–35. <https://doi.org/10.1177/0735633118794515>
 22. Huang T-C, Chen C-C, Chou Y-W. Animating eco-education via augmented reality. *Comput Educ.* 2016;96:72–82. <https://doi.org/10.1016/j.compedu.2016.02.008>
 23. Huang T-C, Chen C-C, Hsu W-P. Do learning styles matter? *Educ Technol Soc.* 2019;22(1):70–81.
 24. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* 2021;372:n71. <https://doi.org/10.1136/bmj.n71>
 25. Hong QN, Pluye P, Fàbregues S, Bartlett G, Boardman F, Cargo M, et al. Mixed Methods Appraisal Tool (MMAT), version 2018. *Educ Inf.* 2018;34(4):285–91. <https://doi.org/10.3233/EFI-180221>
 26. Popay J, Roberts H, Sowden A, Petticrew M, Arai L, Rodgers M, et al. Guidance on the conduct of narrative synthesis in systematic reviews: a product from the ESRC Methods Programme. 2006. <https://www.lancaster.ac.uk/media/lancaster-university/content-assets/documents/fhm/dhr/chir/NSsynthesisguidanceVersion1-April2006.pdf>
 27. Klingenberg S, Johansen M, Vrang LK, Makransky G. Can virtual reality replace real-life physics labs in high school? *Phys Rev Phys Educ Res.* 2020;16(1):010127. <https://doi.org/10.1103/PhysRevPhysEducRes.16.010127>
 28. Önal NT, Önal N. The effect of augmented reality on the astronomy achievement and interest level of gifted students. *Educ Inf Technol.* 2021;26(4):4573–99. <https://doi.org/10.1007/s10639-021-10474-7>
 29. Chng E, Tan AL, Tan SC. Emerging technologies in STEM education: A review of artificial intelligence and immersive technologies. *J STEM Educ Res.* 2023;6:385–407. <https://doi.org/10.1007/s41979-022-00113-w>
 30. Garzón J. An overview of twenty-five years of augmented reality in education. *Multimodal Technol Interact.* 2021;5(7):37. <https://doi.org/10.3390/mti5070037>
 31. Lin X, Chiu TKF, Luo S, Wang L, Chen Z. The role of a teacher learning community in integrating augmented reality into STEM education. *Teach Teach Educ.* 2024;141:104490. <https://doi.org/10.1016/j.tate.2024.104490>
 32. Mayer RE, editor. *The Cambridge Handbook of Multimedia Learning*. 2nd ed. New York: Cambridge University Press; 2014. <https://doi.org/10.1017/CBO9781139547369>
 33. Matovu H, Ungu DAK, Won M, Tsai C-C, Treagust DF, Mocerino M, et al. Immersive virtual reality for science learning: Design, implementation, and evaluation. *Stud Sci Educ.* 2023;59(2):205–44. <https://doi.org/10.1080/03057267.2022.2124710>

Original Article

Beetroot Fruit Powder Attenuates Cardiotoxicity Induced by Monosodium Glutamate via Inhibition of Oxidative Stress and Inflammation in Rats

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Abstract:

Background: Cardiotoxicity, defined as damage to cardiac muscle resulting from exposure to toxic substances, is a growing concern in both environmental and medical contexts. Monosodium glutamate (MSG), a widely used food additive, has been implicated in cardiac toxicity through mechanisms involving inflammation, oxidative stress, and apoptosis. Beetroot (*Beta vulgaris*), rich in nitrates, betalains, and flavonoids, possesses strong antioxidant and anti-inflammatory properties that may counteract MSG-induced cardiac damage.

Objective: This study investigated the cardioprotective potential of beetroot fruit powder (BFP) against MSG-induced cardiotoxicity in male Wistar rats by evaluating its effects on inflammatory, oxidative, apoptotic, cardiac functional markers, and DNA fragmentation index.

Methods: Fifty male Wistar rats (185–205 g) were randomly divided into five groups: control, MSG-only, MSG + low-dose BFP (0.18 g/kg), MSG + high-dose BFP (0.36 g/kg), and MSG recovery group. MSG was administered orally (6 g/kg) for 21 days. BFP treatments were co-administered with MSG. On day 22, cardiac tissues were harvested and analyzed for inflammatory markers (MPO, NO, CRP, TNF- α , IL-1 β , NF- κ B), oxidative stress markers (MDA, SOD, CAT, GSH, GPx, GST), cardiac enzymes (LDH, SDH, CK, GGT), caspase-3 activity, and DNA fragmentation (TUNEL assay). Histological examination was also performed.

Results: MSG exposure significantly elevated pro-inflammatory cytokines, oxidative stress, caspase-3 activity, and cardiac dysfunction markers, alongside pronounced DNA fragmentation and histological alterations. BFP co-treatment, particularly at the high dose, significantly attenuated these changes by reducing pro-inflammatory markers, restoring antioxidant enzyme levels, normalizing cardiac enzyme activities, and lowering DNA fragmentation index. Histology confirmed structural recovery of cardiac tissue in BFP-treated groups. These results underscore the potential of BFP as a dietary intervention to mitigate chemically induced myocardial injury. While the MSG dose used exceeds typical human exposure, this model provides valuable mechanistic insights. Future studies should explore chronic, lower-dose MSG exposure, gene-level regulatory mechanisms, and translational trials in humans.

Conclusion: Beetroot fruit powder demonstrates a potent cardioprotective effect against MSG-induced toxicity by modulating oxidative, inflammatory, apoptotic, and functional biomarkers. These findings highlight its potential as a natural therapeutic agent for mitigating chemically-induced cardiac injury.

Keywords: Beetroot Fruit Powder; Monosodium Glutamate; Cardiotoxicity; Oxidative Stress; Inflammation

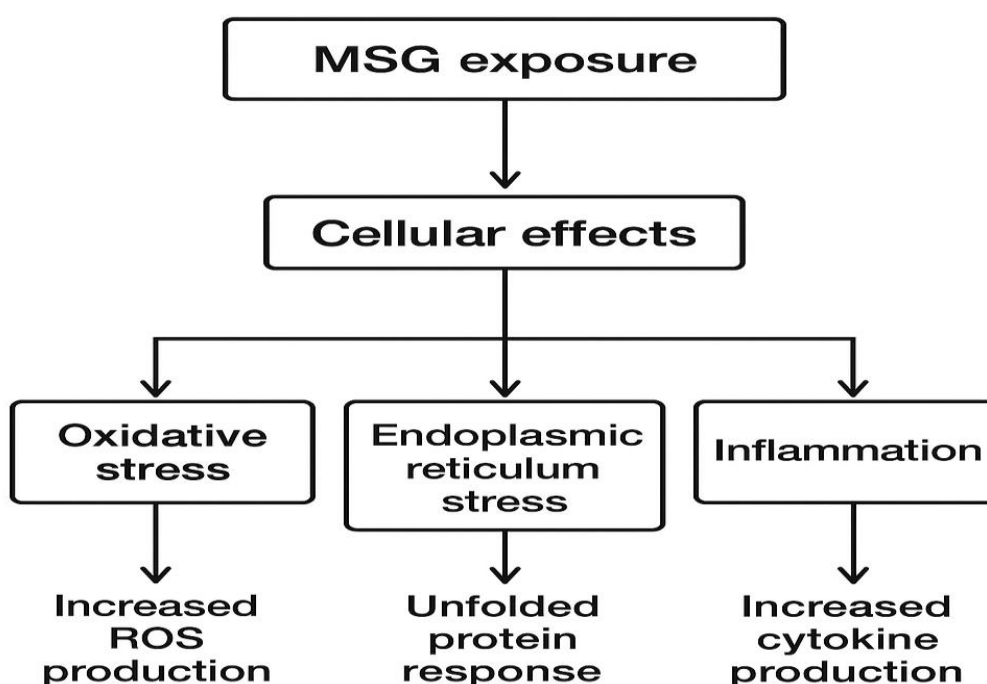
Introduction

Cardiotoxicity, defined as damage to the heart muscle caused by exposure to hazardous substances, is a growing concern in both environmental and medical contexts. It frequently manifests as inflammation, oxidative stress, and apoptotic damage, resulting in structural remodeling and impaired cardiac function.¹ Among the various chemicals implicated, dietary additives such as monosodium glutamate (MSG) have received increased scientific interest due to their potential participation in organ-specific and systemic toxicity, including adverse effects on the cardiovascular system.

MSG, although extensively used as a flavor enhancer and usually viewed as safe, has been documented to disturb redox equilibrium, raise pro-inflammatory cytokine levels, and impair mitochondrial function in various organs, including the heart.² High-dose MSG exposure has been experimentally associated with increased production of nuclear factor kappa B (NF- κ B), myeloperoxidase (MPO), nitric oxide (NO), tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and C-reactive protein (CRP), all of which contribute to inflammation-induced cardiac damage. Furthermore, endogenous antioxidants, including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), are usually decreased, whereas oxidative indicators like malondialdehyde (MDA) are usually increased.³ These abnormalities can cause DNA fragmentation, a sign of permanent cell damage, and trigger apoptotic pathways via caspase-3. Moreover, the release of

enzymes like succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) is a sign of compromised heart function and integrity.⁴

Given the accumulating evidence of MSG-induced cardiotoxicity, it is critical to find effective, natural treatments that might prevent or mitigate these detrimental consequences. The broad-spectrum bioactivity of beetroot (*Beta vulgaris*), a functional food rich in phytochemicals such as betalains, dietary nitrates, flavonoids, and polyphenols, has drawn researchers' curiosity. According to Clifford et al,⁵ beetroot has been shown to reduce pro-inflammatory pathways, oxidative damage, and enhance nitric oxide bioavailability. It has been demonstrated to benefit cardiovascular health in models of metabolic syndrome, ischemia-reperfusion injury, and hypertension. Despite these positive properties, little is known regarding its potential protective role against chemically induced cardiotoxicity, particularly when MSG exposure is present. The purpose of this study is to assess the cardioprotective benefits of beetroot fruit powder in male Wistar rats who have been exposed to MSG. It specifically explores the extent to which beetroot reduces cardiac inflammation, oxidative stress, apoptosis, DNA fragmentation, and dysfunction using biochemical, molecular, and histopathological techniques. This study fills a vacuum in the present knowledge while also contributing to the growing field of natural therapies in toxicology and cardiovascular health management.



Materials

Chemicals and Drugs

All chemicals and drugs utilized in this study were of high-quality analytical grade. Beetroot Fruit Powder was purchased from Simply Precious Enterprise, Badagry, Lagos, Nigeria. Monosodium Glutamate (MSG) was purchased from Mich Mikedenson Nigeria Enterprises Limited, Ilorin, Nigeria. Ethanol, distilled water, and phosphate buffer were acquired from the Laboratory at the Physiology department at Ekiti State University (EKSU), Ado-Ekiti, Nigeria.

Animals

The study was carried out at the Central Animal house at EKSU. A total of fifty male Wistar rats weighing between 185 - 205kg were obtained from the same Central Animal House. The animals were kept under standard laboratory conditions and housed in well-aerated plastic cages with aluminium cover bedded with saw dust. They were fed with tap water and quality rat pellets (Tripods feed limited, Nigeria) on ad libitum. The research protocol approval was obtained from the Department of Physiology's Research Ethical Committee at Ekiti State University with the ethical number, EKSU/P100/2024/01/002.

Stock Solution Preparation

Throughout the study, MSG was administered at a dosage of 6 grams per kilogram (6 g/kg) (The stock solution was prepared by dissolving 0.6 grams of MSG

in one milliliter (1 ml) of distilled water, with a concentration of 600 mg/mL. BFP was administered orally at two dosage levels: a low dose (0.18 g/kg body weight) and a high dose (0.36 g/kg body weight). The stock solutions were prepared by dissolving 0.18 g and 0.36 g of BFP in 1 mL of distilled water, resulting in concentrations of 180 mg/mL and 360 mg/mL, respectively. These doses were selected based on preliminary pilot testing and modified from dose ranges reported in previous studies investigating the physiological and therapeutic effects of beetroot powder in animal models. While literature reports vary widely in dosing, from 200 to 1000 mg/kg depending on the route, duration, and experimental model, the doses in this study were chosen to allow the evaluation of both sub-therapeutic and moderately therapeutic effects over a short-term exposure window. BFP was freshly prepared daily and administered via oral gavage for 21 consecutive days.⁷⁻⁸

Experimental Protocol

After acclimating the animals to the laboratory environment for fourteen days, the 50 male Wistar rats were randomly grouped into five groups of ten animals each (A, B, C, D, and E). Administration was done daily through oral routes for twenty-one days, and the animals were treated accordingly using oral cannulas as shown in Table I.

Table I: Experimental Grouping

Groups	Treatments	Dosage
Group 1	Negative control (distilled water)	1 ml/100 0kg body weight
Group 2	Positive control (MSG only)	0.6 g/100 g body weight
Group 3	MSG + BFP Low dose	0.18g/100g body weight
Group 4	MSG + BFP High dose	0.36g/100g bodyweight
Group 5	Recovery group	(MSG only and left for another 21 days after administration)

Sacrifice and Specimen/Tissue Collection

On day 22, 24 hours after the last dosage, the rats were weighed and culled with an intraperitoneal injection of ketamine-xylene (40mg/kg - 4mg/kg), as previously reported by Ajayi and Akhigbe,⁹ the hearts were collected, weighed, and recorded as previously documented. Blood samples were collected from the heart and centrifuged at 3000/RMP for 15 minutes to separate the serum from the clot. A portion of the heart was homogenized with a laboratory mortar and pestle in freshly produced phosphate buffer solution (pH 7.4), and the homogenate was kept at 800°C for biochemical analysis. The remaining piece of the heart was preserved in a vial with 10% neutral buffer formalin for histological examination.¹⁰

Biochemical Evaluation

Myeloperoxidase (MPO) Activity

Cardiac MPO activity was assessed according to the modified protocol of Goiffon et al.¹¹ Approximately 50 mg of heart tissue was homogenized in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB). After sonication and freeze-thaw cycles, the homogenate was centrifuged at 15,000 × g for 15 minutes at 4°C. The supernatant was incubated with o-dianisidine dihydrochloride and H₂O₂, and absorbance was measured at 460 nm. MPO activity was expressed as units per milligram of protein.

Estimation of Nitric Oxide (NO)

Nitric oxide production was determined by measuring its stable metabolite, nitrite, using the Griess reaction as described by Vargas-Maya et al.¹² Tissue homogenates were deproteinized and incubated with equal volumes of 1% sulfanilamide and 0.1% N-(1-naphthyl)ethylenediamine in 2.5% phosphoric acid. The absorbance of the pink azo dye was read at 540 nm. Nitrite concentrations were calculated using a sodium nitrite standard curve.

C-Reactive Protein (CRP) Analysis

CRP levels in cardiac tissue homogenates were quantified using a high-sensitivity ELISA kit (Rat CRP ELISA Kit, e.g., Elabscience), following the manufacturer's instructions. Homogenates were added to ELISA plate wells pre-coated with anti-rat CRP antibodies. After incubation, substrate solution was added, and absorbance was measured at 450 nm. Concentrations were derived from the standard curve.

Determination of Tumor Necrosis Factor-alpha (TNF- α)

The electrochemical bioassay using Affibody®, a non-immunoglobulin protein, effectively detects TNF- levels in serum samples, with a detection limit of 38 pg/mL and a quantification range of 76-5000 pg/mL.¹³

Determination of Interleukin-1 Beta (IL-1 β)

IL-1 β concentration in cardiac tissue was quantified using a rat IL-1 β ELISA kit as per the manufacturer's instructions. Standards and samples were incubated in antibody-coated wells, followed by detection antibodies and substrate development. Absorbance was measured at 450 nm.

Nuclear Factor kappa B (NF- κ B) Activity

NF- κ B p65 levels were evaluated using a rat-specific NF- κ B ELISA kit. Cardiac tissue homogenates were prepared according to the kit protocol. After sequential incubation with detection antibodies and HRP conjugates, absorbance was recorded at 450 nm.

Malondialdehyde (MDA) Analysis

MDA, a marker of lipid peroxidation, was measured using the thiobarbituric acid reactive substances (TBARS) method, as adapted from Tsikas.¹⁴ Briefly, cardiac homogenates were mixed with thiobarbituric acid (TBA) and acetic acid, heated at 95°C for 60 minutes, and then cooled. The pink chromogen formed was extracted in butanol and measured at 532 nm. MDA concentration was expressed as nmol/mg protein using a standard curve of 1,1,3,3-tetramethoxypropane.

Superoxide Dismutase (SOD) Activity

SOD activity was measured based on its ability to inhibit the auto-oxidation of pyrogallol, following the method as modified by Islam et al.¹⁵ Tissue

homogenates were incubated with Tris-HCl buffer (pH 8.2) and pyrogallol. The rate of auto-oxidation was monitored at 420 nm, and one unit of SOD activity was defined as the amount causing 50% inhibition.

Catalase (CAT) Activity

Catalase activity was determined using the method of Afsar et al.¹⁶ In brief, cardiac homogenates were added to a solution containing 30 mM hydrogen peroxide in phosphate buffer (pH 7.0). The decomposition of H₂O₂ was monitored at 240 nm for 60 seconds. Results were expressed as μ mol of H₂O₂ decomposed/min/mg protein.

Estimation of Glutathione (GSH)

GSH was estimated using Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB). Cardiac tissue was homogenized in 5% sulfosalicylic acid and centrifuged. Supernatants were reacted with DTNB, and the absorbance of the yellow chromophore was measured at 412 nm. GSH levels were expressed as μ mol/g tissue.¹⁷

Estimation of Glutathione Peroxidase (GPx)

GPx activity was evaluated using the NADPH-coupled method as described by Schwarz et al.¹⁸ Cardiac homogenates were incubated with hydrogen peroxide, glutathione, glutathione reductase, and NADPH. The decrease in absorbance at 340 nm due to NADPH oxidation was monitored. Activity was expressed as U/mg protein.

Glutathione-S-Transferase (GST) Activity

GST activity was determined spectrophotometrically using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. Cardiac homogenates were incubated with CDNB and reduced GSH, and the conjugation reaction was monitored at 340 nm. The procedure was based on the method adapted by Robin et al.¹⁹

Determination of Lactate Dehydrogenase (LDH)

LDH activity in cardiac tissue was measured using the method of Klein et al.²⁰ based on the oxidation of NADH to NAD⁺ during the conversion of pyruvate to lactate. Cardiac homogenates were incubated with a reaction mixture containing 100 mM sodium phosphate buffer (pH 7.4), 0.15 mM NADH, and 1.5 mM pyruvate. The decrease in absorbance was recorded at 340 nm for 3 minutes. LDH activity was expressed as units per milligram of protein.

Determination of Succinate Dehydrogenase (SDH)

SDH activity was determined by monitoring the reduction of 2,6-dichlorophenolindophenol (DCPIP) as described by Moreno et al.²¹ Heart tissue homogenates were incubated with a reaction mixture containing sodium succinate (20 mM), potassium

phosphate buffer (pH 7.4), and DCPIP (40 μ M). The decrease in absorbance at 600 nm was recorded. SDH activity was expressed in μ mol/min/mg protein.

Creatine Kinase (CK) Analysis

CK activity was evaluated using a commercial CK assay kit or buffer containing creatine phosphate and ADP. CK catalyzes the conversion of creatine phosphate and ADP to creatine and ATP, coupled with a colorimetric detection of NADH consumption via hexokinase and glucose-6-phosphate dehydrogenase reactions. Absorbance was measured at 340 nm, and results were expressed as U/L.

Gamma-glutamyl Transferase (GGT) Activity

As Jiang et al²² described, GGT activity was measured spectrophotometrically using γ -glutamyl-p-nitroanilide as a substrate and glycylglycine as an acceptor. The formation of p-nitroaniline was measured at 405 nm. Results were expressed as units per liter (U/L) of tissue homogenate.

Caspase-3 Activity Assay

Caspase-3 activity in cardiac tissue was measured using a colorimetric assay based on the cleavage of the chromogenic substrate Ac-DEVD-pNA, following the method of Wynne and Elmes.²³ Briefly, cardiac homogenates were prepared in lysis buffer and incubated with the substrate at 37 °C for 2 hours. The release of p-nitroaniline (pNA) was quantified by measuring absorbance at 405 nm. Caspase-3 activity was expressed as units per mg of protein.

DNA Fragmentation Index (TUNEL Assay)

Cardiac DNA fragmentation was assessed using the TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling) assay according to the protocol adapted by Sharma et al.²⁴ Paraffin-embedded cardiac sections were deparaffinized, rehydrated, and treated with proteinase K. DNA strand breaks were labeled with fluorescein-conjugated dUTP in the presence of terminal deoxynucleotidyl transferase (TdT). TUNEL-positive nuclei were visualized using fluorescence microscopy, and DNA fragmentation index (DFI) was expressed as the percentage of TUNEL-positive cells per field.

Histopathological Analysis

The collected hearts were fixed in Bouin's solution, dehydrated using an increasing ethanol series, and finally cleaned with toluene. The heart was imbedded at ambient temperature, then blocked in paraffin wax and kept in a 60°C incubator overnight. Hematoxylin and eosin (H&E) staining was performed on 5 μ m thick paraffin slices of the heart. The stained slides were inspected using a light microscope, and photomicrographs were obtained at 100x and 400x magnifications.²⁵

Statistical Analysis

The data was analyzed using the GraphPad Prism software (version 9.0, GraphPad Software, Inc.). Data are presented as mean $\pm$ SD. For multiple group comparisons, one-way ANOVA was used, followed by the Tukey post hoc test. P-values <0.05 were considered statistically significant.

Results

Effect of BFP on body weight and heart weight in MSG exposed rats

Table II depicted the results of the effect of BFP on IBW, FBW, BWC, and HW in MSG exposed rats. The IBW and FBW showed no significant ($p>0.05$) difference across all the groups. The BWC revealed a significant ($p<0.05$) decrease in group 2 rats and an increase in group 4 rats when compared with control rats, a significant ($p<0.05$) increase in BWC of rats in groups 3,

4, and 5 when compared with group 2 rats were also observed, a significant ($p<0.05$) increase in group 4 rats, and a decrease in group 5 rats BWC when compared with groups 3 and 4 rats, respectively. Meanwhile, the HW showed a significant ($p<0.05$) decrease in groups 2, 3, and 5 rats when compared with group 1 rats. Besides, the HW also expressed a significant ($p<0.05$) increase in groups 4 and 5 rats when compared with group 2 rats.

Table II: Effect of BFP on Body weight and heart weight in rats exposed to MSG

	Groups				
	Group 1 (Control)	Group 2 (MSG-exposed)	Group 3 (MSG-exposed+LD BFP)	Group 4 (MSG-exposed+HD BFP)	Group 5 (MSG-exposed-R)
IBW (g)	193.00 $\pm$ 10.58	199.7 $\pm$ 7.57	194.7 $\pm$ 5.13	188.7 $\pm$ 3.51	193.00 $\pm$ 3.61

FBW (g)		210.30 ± 7.64	217.30 ± 6.43	217.00 ± 2.00	215.00 ± 4.583
	216.70 ± 11.93				
BWC (g)	23.67 ± 1.53	10.67 ± 1.53 ^a	22.67 ± 1.53 ^b	28.33 ± 1.53 ^{abc}	22.00 ± 1.00 ^{bd}
HW (g)	1.27 ± 0.12	0.83 ± 0.06 ^a	0.97 ± 0.06 ^a	1.23 ± 0.06 ^{bc}	1.07 ± 0.06 ^{ab}

Values are mean ± SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs MSG-exposed, c $P < 0.05$ vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-

exposed+HD BFP, and IBW, FBW, BWC, and HW were initial body weight, final body weight, body weight change, and heart weight, respectively.

Effect of BFP on Cardiac MPO, NO, and CRP in MSG exposed rats

The results of cardiac MPO, NO, and CRP were as expressed in Figure 1. The result of cardiac MPO (Figure 1A) showed a significant ($p < 0.05$) increase in groups 2, 3, and 5 rats when compared with the control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with the group 2 rats, and a significant ($p < 0.05$) decrease in groups 4 and 5 rats when compared with group 3 rats. The result of cardiac NO (Figure 1B) revealed a significant ($p < 0.05$) increase

in groups 2 and 3 rats when compared with the control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with the group 2 rats, a significant ($p < 0.05$) decrease in group 4 rats when compared with group 3 rats, and a significant ($p < 0.05$) increase in group 5 rats when compared with group 4 rats, while the result of cardiac CRP (Figure 1C) depicted a significant ($p < 0.05$) increase in group 2 rats when compared with the control rats and a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with the group 2 rats.

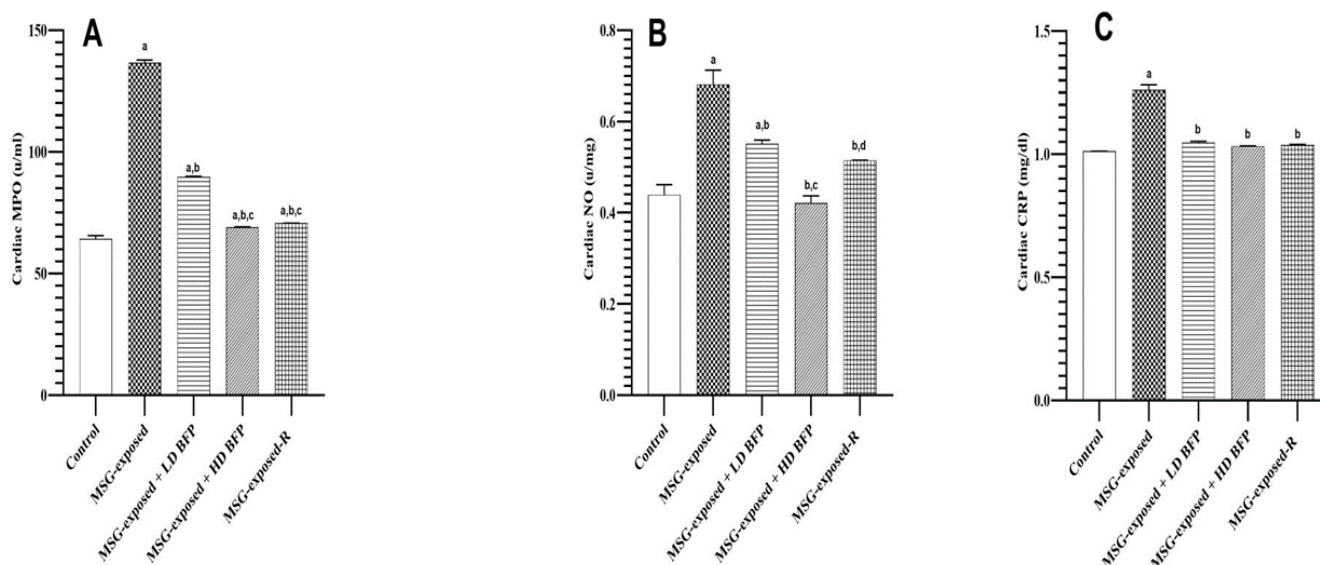


Fig 1 Effect of BFP on Cardiac MPO, NO, and CRP in MSG exposed rats. Values are mean ± SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs

MSG-exposed, c $P < 0.05$ vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-exposed+HD BFP.

Effect of BFP on Cardiac IL-1 β , TNF- α , and NF-k β in MSG exposed rats

The results of cardiac IL-1 β , TNF- α , and NF-k β were as expressed in Figure 2. The result of cardiac IL-1 β and TNF- α (Figure 2A and B) showed a significant ($p < 0.05$) increase in groups 2, 3, 4, and 5 rats when

compared with the control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with the group 2 rats, a significant ($p < 0.05$) decrease in groups 4 and 5 rats when compared with the group 3 rats, and a significant ($p < 0.05$) decrease in group 5 rats when compared with the group 4 rats. The result of

cardiac NF- κ B (Figure 2C) revealed a significant ($p<0.05$) increase in group 2, 3, 4, and 5 rats when compared with the control rats, a significant ($p<0.05$) decrease in groups 3, 4, and 5 rats when compared with

group 2 rats, and a significant ($p<0.05$) decrease in groups 4 and 5 rats when compared with the group 3 rats.

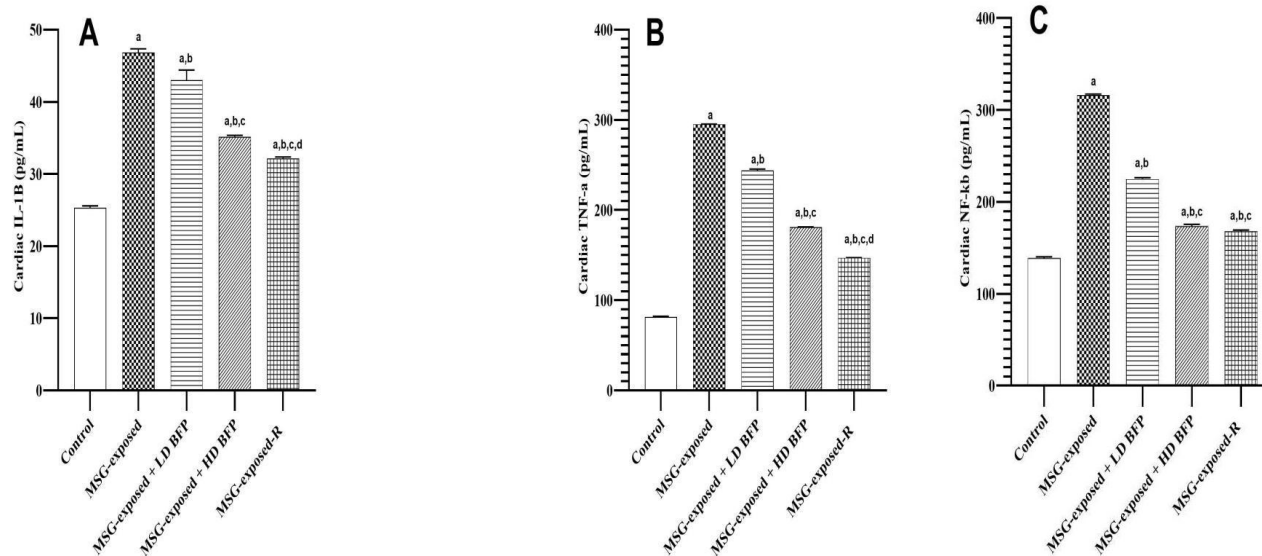


Figure 2: Effect of BFP on Cardiac IL-1 β , TNF- α , and NF- κ B in MSG exposed rats.

Values are mean $\pm$ SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs MSG-exposed, c $P < 0.05$

vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-exposed+HD BFP.

Effect of BFP on Cardiac MDA, CAT, and SOD in MSG exposed rats

The results of cardiac MDA, CAT, and SOD were as expressed in Figure 3. The result of cardiac MDA (Figure 3A) showed a significant ($p<0.05$) increase in groups 2, 3, 4, and 5 rats when compared with the control rats, a significant ($p<0.05$) decrease in groups 3, 4, and 5 rats when compared with group 2 rats, and a significant ($p<0.05$) decrease in groups 4 and 5 rats when compared with group 3 rats. The result of cardiac

CAT (Figure 3B) revealed a significant ($p<0.05$) decrease in groups 2, 3, 4, and 5 rats when compared with the control rats, a significant ($p<0.05$) increase in groups 3, 4, and 5 rats when compared with group 2 rats, and a significant ($p<0.05$) increase in groups 4 and 5 rats when compared with group 3 rats, while SOD (Figure 3C) result depicted a significant ($p<0.05$) decrease in groups 2, 3, 4, and 5 rats when compared with the control rats and a significant ($p<0.05$) increase in groups 4 and 5 rats when compared with groups 2 and 3 rats.

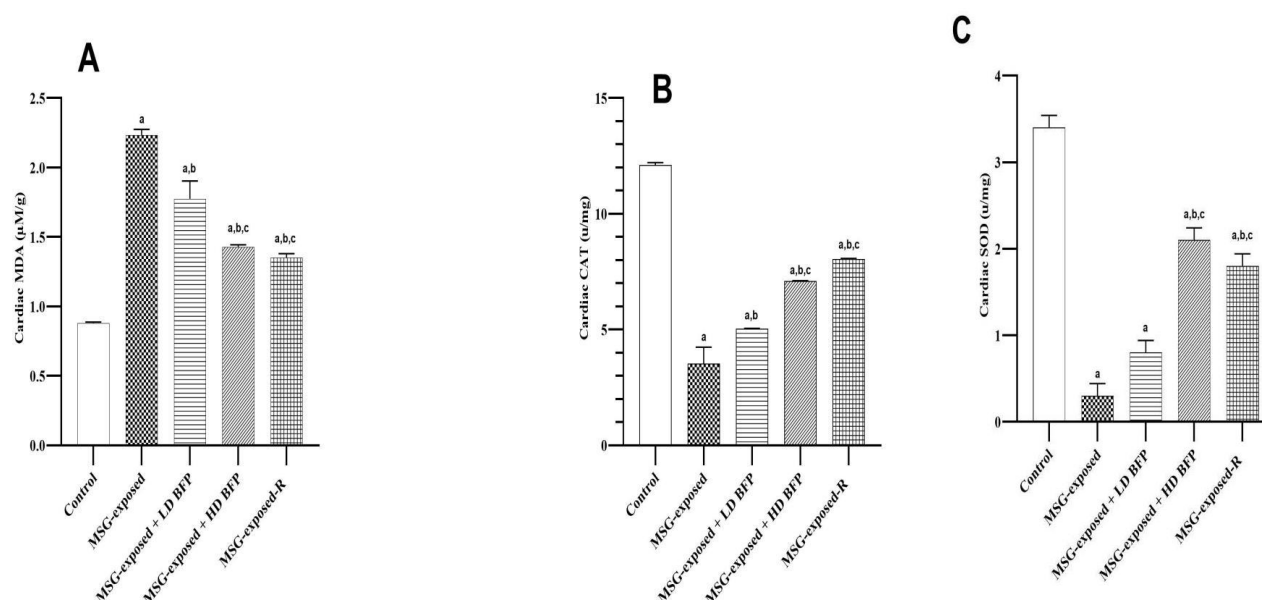


Figure 3: Effect of BFP on Cardiac MDA, CAT, and SOD in MSG exposed rats.

Values are mean $\pm$ SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs MSG-exposed, c $P < 0.05$

vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-exposed+HD BFP.

Effect of BFP on Cardiac GSH, GST, and GPx in MSG exposed rats

The results of cardiac GSH, GST, and GPx were as expressed in Figure 4. The result of cardiac GSH (Figure 4A) showed a significant ($p < 0.05$) decrease in groups 2, 3, 4, and 5 rats when compared with the control rats, a significant ($p < 0.05$) increase in groups 4 and 5 rats when compared with the group 2 rats, a significant ($p < 0.05$) increase in group 4 rats when compared with the group 3 rats, and a significant ($p < 0.05$) decrease in group 5 rats when compared with the group 4 rats. The result of cardiac GST (Figure 4B)

revealed a significant ($p < 0.05$) decrease in group 2, 3, 4, and 5 rats when compared with the control rats, and a significant ($p < 0.05$) increase in groups 3, 4, and 5 rats when compared with the group 2 rats, while the result of cardiac GPx (Figure 4C) showed a significant ($p < 0.05$) decrease in group 2, 3, 4, and 5 rats when compared with the control rats, a significant ($p < 0.05$) increase in groups 3, 4, and 5 rats when compared with the group 2 rats, a significant ($p < 0.05$) increase in groups 4 and 5 rats when compared with group 3 rats, and a significant ($p < 0.05$) increase in group 5 rats when compared with the group 4 rats.

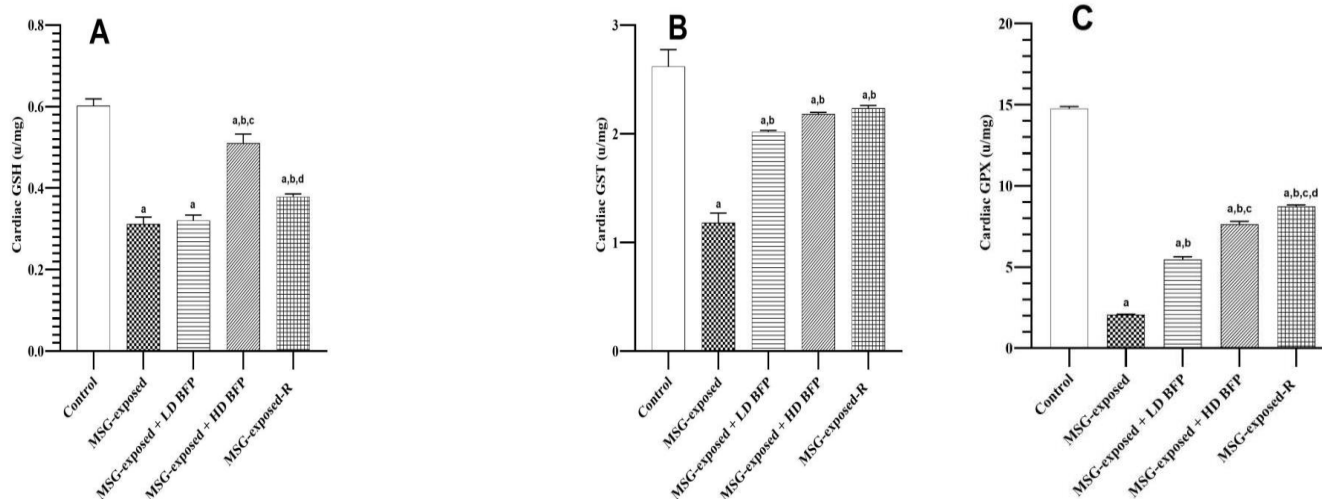


Figure 4: Effect of BFP on Cardiac GSH, GST, and GPx in MSG exposed rats.

Values are mean $\pm$ SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs MSG-exposed, c $P < 0.05$

vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-exposed+HD BFP.

Effect of BFP on Heart Functional markers in MSG exposed rats

The results of heart functions were as expressed in Figure 5. The result of SDH (Figure 5A) showed a significant ($p < 0.05$) increase in groups 2, 3, 4, and 5 rats when compared with the control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with group 2 rats, and a significant ($p < 0.05$) increase in group 4 rats when compared with group 3 rats. The results of LDH and creatinine kinase (Figure 5B and D) showed a significant ($p < 0.05$) increase in groups 2, 3, 4, and 5 rats when compared with the

control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with group 2 rats, and a significant ($p < 0.05$) decrease in groups 4 and 5 rats when compared with group 3 rats. The result of GGT (Figure 5C) showed a significant ($p < 0.05$) increase in groups 2, 3, 4, and 5 rats when compared with the control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with group 2 rats, a significant ($p < 0.05$) decrease in group 4 rats when compared with group 3 rats, and a significant ($p < 0.05$) increase in group 5 rats when compared with group 4 rats.

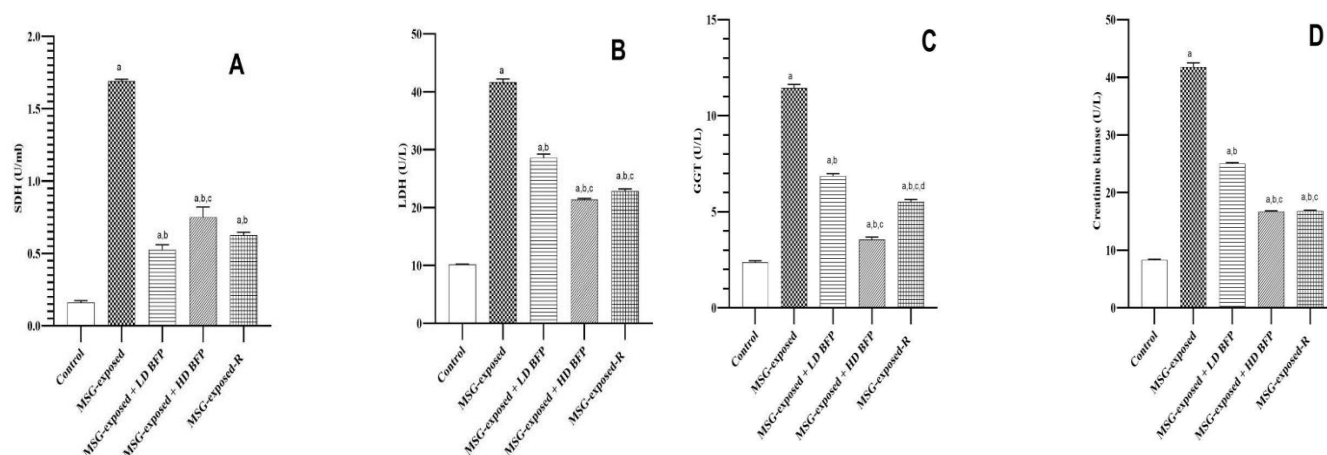


Figure 5: Effect of BFP on Heart Functional Markers in MSG exposed rats.

Values are mean $\pm$ SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs MSG-exposed, c $P < 0.05$

vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-exposed+HD BFP.

Effect of BFP on Heart Caspase-3 in MSG exposed rats

The results of heart caspase-3 was as expressed in Figure 6. The result of caspase-3 showed a significant ($p < 0.05$) increase in groups 2, 3, and 5 rats when

compared with the control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with group 2 rats, and a significant ($p < 0.05$) decrease in groups 4 and 5 rats when compared with group 3 rats.

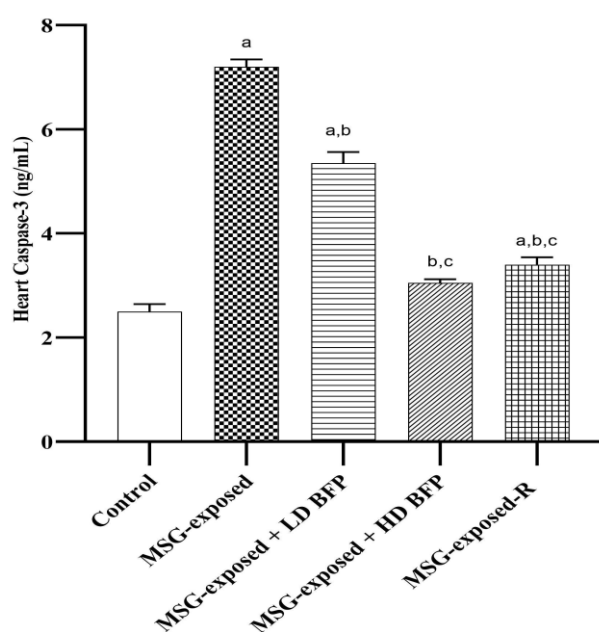


Figure 6: Effect of BFP on Heart caspase-3 in MSG exposed rats. Values are mean $\pm$ SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs

MSG-exposed, c $P < 0.05$ vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-exposed+HD BFP.

Effect of BFP on Cardiac DFI in MSG Exposed Rats

The results of cardiac DFI was as expressed in Figure 7. The result of DFI revealed a significant ($p < 0.05$) increase in groups 2, 3, and 5 rats when

compared with the control rats, a significant ($p < 0.05$) decrease in groups 3, 4, and 5 rats when compared with group 2 rats, a significant ($p < 0.05$) decrease in groups 4 and 5 rats when compared with group 3 rats, and a

significant ($p < 0.05$) increase in group 5 rats when compared with group 4 rats.

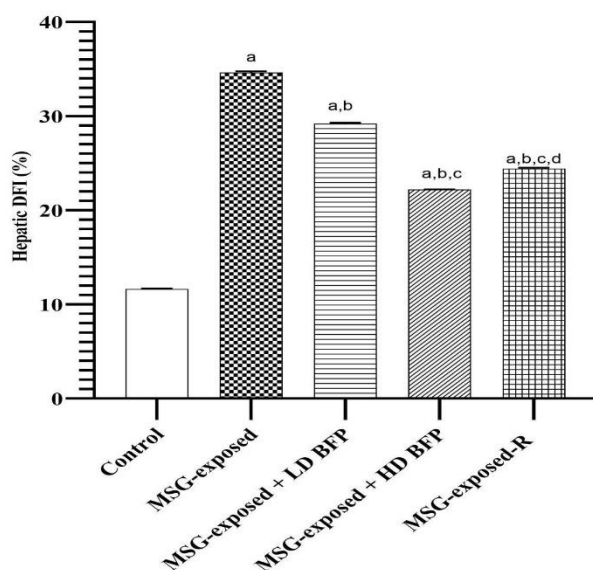


Figure 7: Effect of BFP on Cardiac DFI in MSG exposed rats.

Values are mean $\pm$ SD of 3 replicates, where a $P < 0.05$ vs control, b $P < 0.05$ vs MSG-exposed, c $P < 0.05$

vs MSG-exposed+LD BFP, and d $P < 0.05$ vs MSG-exposed+HD BFP.

Effect of BFP on Cardiac Histology in MSG exposed rats

Photomicrograph (Figure 8A) shows the heart tissue composed predominantly of the cardiac parenchyma and portal regions. The cardiac cells (C) appear polygonal and are disposed in sheet with a well outlined nucleus (N) the cardiac cells are separated by the sinusoids (S) with thin endothelial lining. The portal region (circle), composed of branches of the Cardiac portal vessels appear normal implying normal histomorphology of the heart tissue.

Photomicrograph (Figure 8B) shows the heart tissue composed predominantly of the cardiac parenchymal and portal regions. The cardiac cells (C) appear polygonal and are disposed in sheet with a well outlined nucleus (N). The cardiac cells are separated by the sinusoids (S) with thin endothelial). The portal region (circle), composed of branches of the Cardiac portal vessels (CPV), and showed vascular congestion (Star) and mild inflammatory cell infiltration (arrow head) implying inflammation of the heart tissue

Photomicrograph (Figure 8C) shows the heart tissue composed predominantly of the cardiac parenchymal and portal regions. The cardiac cells (C) appear polygonal and are disposed in sheet with a well

outlined nucleus (N). The cardiac cells are separated by the sinusoids (S) with thin endothelial lining. The portal region (circle), composed of branches of the Cardiac portal vessels (CPV), and showed moderate inflammatory cell infiltration implying periportal inflammation of the heart tissue.

Photomicrograph (Figure 8D) shows the heart tissue composed predominantly of the cardiac parenchyma and portal regions. The cardiac cells (C) appear polygonal and are disposed in sheet with a well outlined nucleus (N) the cardiac cells are separated by the sinusoids (S) with thin endothelial lining. The portal region (circle), composed of branches of the cardiac portal vessels appear normal implying normal histomorphology of the heart tissue.

Photomicrograph (Figure 8E) shows the heart tissue composed predominantly of the cardiac parenchymal and portal regions. The cardiac cells (C) appear polygonal and are disposed in sheet with a well outlined nucleus (N). The cardiac cells are separated by the sinusoids (S) with thin endothelial lining. The portal region (circle), composed of branches of the cardiac portal vessels (CPV), and showed mild inflammatory cell infiltration (arrow head) implying cardiac cellular reaction to injury.

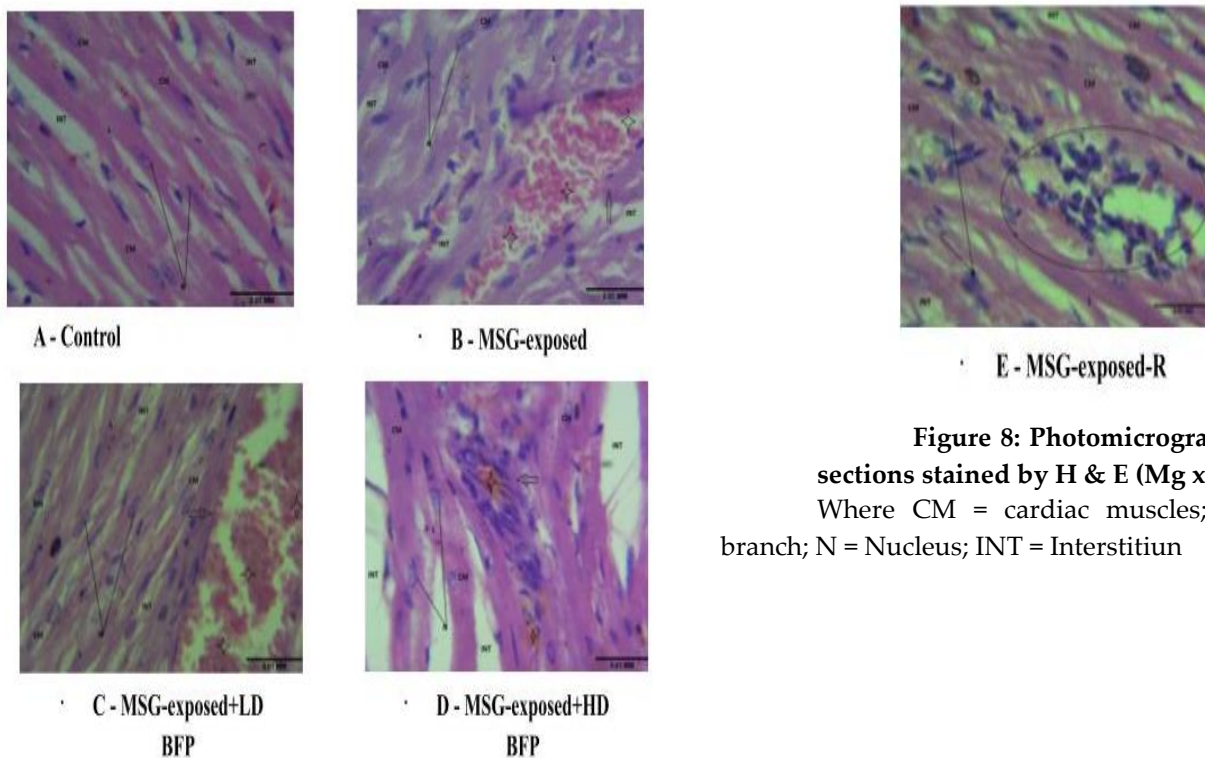


Figure 8: Photomicrograph of cardiac sections stained by H & E (Mg x400).

Where CM = cardiac muscles; L = Lateral branch; N = Nucleus; INT = Interstitium

Discussion

BFP, a functional food rich in phytochemicals such as betalains, flavonoids, and dietary nitrates, has received a lot of interest for its cardioprotective properties. In this work, BFP significantly reduced the biochemical, molecular, and histological changes caused by monosodium glutamate (MSG), a food toxicant that causes systemic oxidative and inflammatory stress. The observed effects indicate that BFP exerts its cardioprotective impact by influencing critical cellular pathways involved in oxidative stress, inflammation, apoptosis, and DNA damage, protecting cardiac structure and function.

The cardiotoxic effects of MSG were confirmed through the significant elevation of pro-inflammatory markers including MPO, NO, CRP, IL-1 β , TNF- α , and NF- κ B in MSG-exposed rats compared with controls. These cytokines and signaling mediators are key orchestrators of inflammatory responses that initiate and sustain myocardial injury.²⁶ Persistent activation of NF- κ B promotes the transcription of several pro-inflammatory genes, amplifying cardiac damage. The significant reduction of these markers following BFP administration, especially at higher doses, indicates that BFP mitigates inflammation by inhibiting NF- κ B-dependent transcription and scavenging reactive free radicals through its polyphenolic and betalain content. These findings align with previous studies that demonstrated the anti-inflammatory potential of *Beta vulgaris* through suppression of NF- κ B signaling and cytokine release.^{5,27}

Oxidative stress also played a central role in MSG-induced cardiotoxicity, as evidenced by elevated malondialdehyde (MDA) levels and decreased antioxidant enzyme activities (SOD, CAT, GSH, GPx, and GST). This pattern indicates excessive reactive oxygen species (ROS) generation, lipid peroxidation, and impaired antioxidant defense systems, consistent with earlier findings on MSG-mediated cardiac injury.² The substantial reduction in MDA levels and restoration of antioxidant enzyme activities in BFP-treated rats suggest potent redox regulatory effects of beetroot bioactives. These effects are likely mediated through activation of the Nrf2 signaling pathway, which upregulates endogenous antioxidant enzymes and enhances cellular resilience to oxidative damage.^{15,27}

Similarly, assessment of cardiac function markers (LDH, SDH, CK, and GGT) revealed pronounced elevations in MSG-treated animals, confirming myocardial cell membrane disruption and enzymatic leakage. Treatment with BFP significantly reduced these enzyme levels, particularly at higher doses, suggesting stabilization of cardiomyocyte membranes and improved mitochondrial integrity. This protective response corroborates previous evidence that natural antioxidants can prevent enzyme leakage and limit necrotic cell death in cardiotoxicity models.²⁸

Apoptosis, a key downstream effect of oxidative and inflammatory stress, was evident in the

elevated caspase-3 activity observed in MSG-exposed rats. The marked reduction of caspase-3 activity following BFP administration demonstrates the compound's anti-apoptotic potential, possibly achieved through inhibition of mitochondrial cytochrome-c release and suppression of pro-apoptotic signaling cascades. This anti-apoptotic mechanism aligns with beetroot's known ability to modulate intrinsic cell death pathways via its high nitrate and flavonoid content.^{5,29}

In rats exposed to MSG, the DNA fragmentation index (DFI), a measure of genotoxic damage, also increased noticeably. Treatment with BFP decreased DNA fragmentation, which may indicate improved genomic stability and less oxidative DNA damage. The capacity of beetroot phytochemicals to assist DNA repair processes and neutralize reactive species that cause strand breaks may be the cause of this action.³⁰

Histopathological examination further validated the biochemical results. Myocardial sections

from MSG-exposed rats showed severe inflammatory infiltration, vascular congestion, and disorganized myofibrillar architecture—hallmarks of structural damage. Conversely, BFP-treated groups exhibited restored histoarchitecture with reduced inflammatory cell infiltration and preserved nuclei. These findings reinforce the biochemical and molecular evidence of recovery and demonstrate that BFP effectively reverses MSG-induced cardiac remodeling and dysfunction, similar to reports in other models of chemically induced cardiotoxicity.³¹

Collectively, these findings establish that beetroot fruit powder confers comprehensive cardioprotection against MSG-induced injury through integrated antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. Its rich phytochemical composition and ability to restore redox balance suggest potential for its use as a natural therapeutic agent in preventing chemically induced cardiovascular disorders

Conclusion

These findings highlight the potential of beetroot fruit powder as a cardioprotective agent capable of averting chemically induced myocardial damage by suppressing inflammatory and apoptotic signaling, reducing oxidative stress, and stabilizing

cardiac biomarkers. Its phytochemical makeup most likely contributes significantly to these effects, indicating its potential as a natural dietary intervention in the prevention or treatment of cardiac toxicity.

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References

1. Mamoshina P, Rodríguez B, Bueno-Orovio A. Toward a broader view of mechanisms of drug cardiotoxicity. *Cell Rep Med*. 2021;2:100216. doi:[10.1016/j.xcrm.2021.100216](https://doi.org/10.1016/j.xcrm.2021.100216).
2. Hazzaa S, El-Roghy E, Eldaim M, Elgarawany G. Monosodium glutamate induces cardiac toxicity via oxidative stress, fibrosis, and P53 proapoptotic protein expression in rats. *Environ Sci Pollut Res Int*. 2020;27(16):20014-24. doi:[10.1007/s11356-020-08436-6](https://doi.org/10.1007/s11356-020-08436-6).
3. Ogunmokunwa A, Ibitoye B. Monosodium glutamate (MSG) exposure induced oxidative stress and disrupted testicular hormonal regulation, exacerbating reproductive dysfunction in male WISTAR rats. *Endocr Metab Sci*. 2025;:100226. doi:[10.1016/j.endmts.2025.100226](https://doi.org/10.1016/j.endmts.2025.100226).
4. Dai C, Li Q, May H, Li C, Zhang G, Sharma G, et al. Lactate Dehydrogenase A Governs Cardiac Hypertrophic Growth in Response to Hemodynamic Stress. *Cell Rep*.

- 2020;32(8):108087.
doi:[10.1016/j.celrep.2020.108087](https://doi.org/10.1016/j.celrep.2020.108087).
5. Clifford T, Howatson G, West D, Stevenson E. The Potential Benefits of Red Beetroot Supplementation in Health and Disease. *Nutrients*. 2015;7(4):2801-22. doi:[10.3390/nu7042801](https://doi.org/10.3390/nu7042801).
6. Nalugo H, Ninsiima HI, Kasozi KI, Nabirumbi R, Osuwat LO, Matama K, et al. Monosodium Glutamate Maintains Antioxidant Balance in the Neuro-Retinal Axis of Male Wistar Rats. *J Exp Pharmacol*. 2021;13:681-90. doi:<https://doi.org/10.21203/rs.3.rs-508301/v1>.
7. Abd-El-Fattah ME, Dessouki AA, Abdelnaeim NS, Emam BM. Protective effect of Beta vulgaris roots supplementation on anemic phenylhydrazine-intoxicated rats. *Environ Sci Pollut Res Int*. 2021;28(46):65731-42. doi:[10.1007/s11356-021-15302-6](https://doi.org/10.1007/s11356-021-15302-6).
8. Sarfaraz S, Ikram R, Munawwar R, Osama M, Gul S, Sufian M. Rising trend of Nutraceuticals: Evaluation of lyophilized beetroot powder at different doses for its hypolipidemic effects. *Pak J Pharm Sci*. 2021;34(4):1315-22. PMID: 34799303. doi:[10.1186/s40738-020-00074-3](https://doi.org/10.1186/s40738-020-00074-3).
9. Ajayi AF, Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertil Res Pract*. 2020;6:5. doi:[10.1186/s40738-020-00074-3](https://doi.org/10.1186/s40738-020-00074-3).
10. Zaidi AS, Muzaffar M, Gautam S, Alam I. Effect of curcumin on inflammatory and oxidative stress markers in Lipopolysaccharide induced animal models of pre-eclampsia. *Physiology*. 2024 May 1;39(S1):1737. doi:[10.1152/physiol.2024.39.S1.1737](https://doi.org/10.1152/physiol.2024.39.S1.1737).
11. Goiffon RJ, Martinez SC, Piwnica-Worms D. A rapid bioluminescence assay for measuring myeloperoxidase activity in human plasma. *Nat Commun*. 2015;6:6271. doi:[10.1038/ncomms7271](https://doi.org/10.1038/ncomms7271).
12. Vargas-Maya NI, Padilla-Vaca F, Romero-González OE, Rosales-Castillo EAS, Rangel-Serrano Á, Arias-Negrete S, et al. Refinement of the Griess method for measuring nitrite in biological samples. *J Microbiol Methods*. 2021;187:106260. doi:[10.1016/j.mimet.2021.106260](https://doi.org/10.1016/j.mimet.2021.106260).
13. Baydemir G, Bettazzi F, Palchetti I, Voccia D. Strategies for the development of an electrochemical bioassay for TNF-alpha detection by using a non-immunoglobulin bioreceptor. *Talanta*. 2016;151:141-7. doi:[10.1016/j.talanta.2016.01.021](https://doi.org/10.1016/j.talanta.2016.01.021).
14. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Anal Biochem*. 2017;524:13-30. doi:[10.1016/j.ab.2016.10.021](https://doi.org/10.1016/j.ab.2016.10.021).
15. Islam MN, Rauf A, Fahad FI, Emran TB, Mitra S, Olatunde A, et al. Superoxide dismutase: an updated review on its health benefits and industrial applications. *Crit Rev Food Sci Nutr*. 2022;62(26):7282-300. doi:[10.1080/10408398.2021.1913400](https://doi.org/10.1080/10408398.2021.1913400).
16. Afsar T, Razak S, Batoo KM, Khan MR. Acacia hydasypica R. Parker prevents doxorubicin-induced cardiac injury by attenuation of oxidative stress and structural Cardiomyocyte alterations in rats. *BMC Complement Altern Med*. 2017;17(1):554. doi:<https://doi.org/10.1186/s12906-017-2061-0>.
17. Kalinovic S, Stamm P, Oelze M, Daub S, Kröller-Schön S, Kvandová M, et al. Comparison of three methods for in vivo quantification of glutathione in tissues of hypertensive rats. *Free Radic Res*. 2021;55(9-10):1048-61. doi:[10.1080/10715762.2021.2016735](https://doi.org/10.1080/10715762.2021.2016735).
18. Schwarz M, Löser A, Cheng Q, Wichmann-Costaganna M, Schädel P, Werz O, et al. Side-by-side comparison of recombinant human glutathione peroxidases identifies overlapping substrate specificities for soluble hydroperoxides. *Redox Biol*. 2023;59:102593. doi:[10.1016/j.redox.2022.102593](https://doi.org/10.1016/j.redox.2022.102593).
19. Robin SKD, Ansari M, Uppugunduri CRS. Spectrophotometric Screening for Potential Inhibitors of Cytosolic Glutathione S-Transferases. *J Vis Exp*. 2020;(164). doi:[10.3791/61924](https://doi.org/10.3791/61924).
20. Klein R, Nagy O, Tóthová C, Chovanová F. Clinical and Diagnostic Significance of Lactate Dehydrogenase and Its Isoenzymes in Animals. *Vet Med Int*. 2020;2020:5346483. doi:[10.1155/2020/5346483](https://doi.org/10.1155/2020/5346483).
21. Moreno C, Santos RM, Burns R, Zhang WC. Succinate dehydrogenase and ribonucleic acid networks in cancer and other diseases.

- Cancers (Basel). 2020;12(11):3237.
doi:[10.3390/cancers12113237](https://doi.org/10.3390/cancers12113237).
22. Jiang L, Guo D, Wang L, Chang S, Li J, Zhan D, et al. Sensitive and selective SERS probe for detecting the activity of γ -glutamyl transpeptidase in serum. Anal Chim Acta. 2020;1099:119-25. doi:[10.1016/j.aca.2019.11.041](https://doi.org/10.1016/j.aca.2019.11.041).
23. Wynne C, Elmes R. Utilising a 1,8-naphthalimide probe for the ratiometric fluorescent visualisation of caspase-3. Front Chem. 2024;12:1418378.
doi:[10.3389/fchem.2024.1418378](https://doi.org/10.3389/fchem.2024.1418378).
24. Sharma R, Ahmad G, Esteves SC, Agarwal A. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using bench top flow cytometer for evaluation of sperm DNA fragmentation in fertility laboratories: protocol, reference values, and quality control. J Assist Reprod Genet. 2016;33(2):291-300.
doi:[10.1007/s10815-015-0635-7](https://doi.org/10.1007/s10815-015-0635-7).
25. Sridharan D, Pracha N, Dougherty JA, Akhtar A, Alvi SB, Khan M. A one-stop protocol to assess myocardial fibrosis in frozen and paraffin sections. Methods Protoc. 2022;5(1):13.
doi:[10.3390/mps5010013](https://doi.org/10.3390/mps5010013).
26. Reina-Couto M, Pereira-Terra P, Quelhas-Santos J, Silva-Pereira C, Albino-Teixeira A, Sousa T. Inflammation in human heart failure: major mediators and therapeutic targets. Front Physiol. 2021;12:746494.
doi:[10.3389/fphys.2021.746494](https://doi.org/10.3389/fphys.2021.746494).
27. Brzezińska-Rojek J, Sagatovych S, Malinowska P, Gadaj K, Prokopowicz M, Grembecka M. Antioxidant capacity, nitrite and nitrate content in beetroot-based dietary supplements. Foods. 2023;12(5):1017.
doi:[10.3390/foods12051017](https://doi.org/10.3390/foods12051017).
28. Bodor GS. Biochemical markers of myocardial damage. EJIFCC. 2016;27(2):95-111. PMID: 27683527.
29. Tan ML, Hamid SBS. Beetroot as a potential functional food for cancer chemoprevention, a narrative review. J Cancer Prev. 2021;26(1):1-17. doi:[10.15430/JCP.2021.26.1.1](https://doi.org/10.15430/JCP.2021.26.1.1).
30. Barnes JL, Zubair M, John K, Poirier MC, Martin FL. Carcinogens and DNA damage. Biochem Soc Trans. 2018;46(5):1213-24.
doi:[10.1042/BST20180519](https://doi.org/10.1042/BST20180519).
31. Banerjee A, Mukherjee S, Maji BK. Monosodium glutamate causes hepato-cardiac derangement in male rats. Hum Exp Toxicol. 2021;40(12_suppl):S359-S369.
doi:[10.1177/09603271211049550](https://doi.org/10.1177/09603271211049550).